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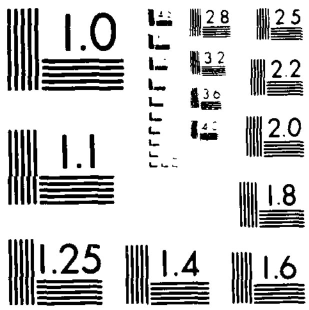

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HUMORAL CONTROL OF REGIONAL BLOOD FLOW IN
HEMORRHAGIC SHOCK IN NON-RESUSCITATED
AND RESUSCITATED ANIMALS

Annual Progress Report

J. Judson McNamara, M.D.

September 1982

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Cardiovascular Research Laboratory
Queen's Medical Center
Honolulu, Hawaii 96813

1983

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20. Abstract

During the past 16 months in experiments in rabbits and primates we have:

1) Reaffirmed a prolonged reduction in blood volume and visceral organ flow for periods of at least 18 hours after resuscitation from hemorrhagic shock.

2) Shown that none of the readily identifiable humoral controls of blood pressure or vascular resistance are involved in this flow redistribution (i.e., renin/angiotensin, catecholamines, thromboxane).

3) Demonstrate improved survival in shocked rabbits treated with ATP-$MgCl_2$.

4) Failed to demonstrate any change in mortality in shocked animals subjected to endorphin antagonism with naloxone.

5) Shown no favorable effect of resuscitation with fluorocarbons on survival in rabbits in hemorrhagic shock.

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Foreword

The present report describes the work we have done during the past 16 months. Some are definitive experiments and others were intended as pilot studies. The structure of the report includes five separate papers describing the five investigations accomplished under the grant this year (Appendices A-E) and the body of the report which summarizes and refers to each appendix. Pertinent bibliographic data is included in each appendix as appropriate.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publicstion No. [NIH]78-23, revised 1978).

Summary

During the past 16 months in experiments in rabbits and primates we have:

1) Reaffirmed a prolonged reduction in blood volume and visceral organ flow for periods of at least 18 hours after resuscitation from hemorrhagic shock.

2) Shown that none of the readily identifiable humoral controls of blood pressure or vascular resistance are involved in this flow redistribution (i.e. renin/angiotensin, cathechol-amines, thromboxane).

3) Demonstrate improved survival in shocked rabbits treated with ATP-$MgCl_2$.

4) Failed to demonstrate any change in mortality in shocked animals subjected to endorphin antagonism with naloxone.

5) Shown no favorable effect of resuscitation with fluoro-carbons on survival in rabbits in hemorrhagic shock.

Body of Report

During the past 16 months a number of projects have been undertaken and completed:

1) We have reassessed our original observations documenting prolonged visceral organ blood flow abnormalities in animals resuscitated from hemorrhagic shock. This is expanded in a preliminary draft of a paper to be presented this September at the American Association for the Surgery of Trauma. The paper is included in Appendix A. The important points are:

a) The reaffirmation of prolonged reduction in blood volume and visceral blood flow in primates successfully resuscitated from hemorrhagic shock.

b) The inadequacy of left atrial pressure as an index of adequate resuscitation.

2) We have now shown that neither catecholamines, renin-angiotensin system nor thromboxane/prostacyclin is involved in the persistance of these blood flow abnormalities as measureable levels of these substances in the serum are all back to normal within 4 hours, whereas the abnormality persists for at least 18 hours. Appendix B.

Furthermore, treatment with aspirin increased mortality significantly. This may be related to the fact that aspirin blocks thromboxane production and possibly for the first few minutes this is important in maintianing viability of the organism and that blocking thromboxane synthesis negatively affects early mortality and morbidity.

3) Using this rabbit model we have looked at the effect of three immediate interventions in survival of rabbits subjected to profound hemorrhagic shock and then resuscitated.

a) ATP-$MgCl_2$ increased survival of rabbits subjected to hemorrhagic shock. Appendix C.

b) Fluosol DA, a fluorocarbon blood substitute, had no effect in survival. Appendix D.

c) Treatment with naloxone. Appendix E.

1. Did not affect rabbits in hemorrhagic shock.

2. Had little affect as did morphine on rabbit hemodynamics.

3. Had profound beneficial effects on one primate studied.

APPENDIX A

Resuscitation From Shock

It has been only 40 years since Blalock[1] and Wiggers[2] led the way to understanding that hemorrhagic shock was failure of the circulatory system to deliver adequate blood flow to the tissue. Therapy initially focused on volume replacement. Resuscitation has been considered the restoration of hemodynamic normality as defined by a number of commonly measured parameters. Nevertheless, in spite of frequent instances of apparently successful initial resuscitation patients continued to die of organ failure apparently as a consequence of the episode of shock.

Improvements in resuscitation have occurred through comprehensive hemodynamic monitoring, a better appreciation of fluid shifts, and more recently understanding some of the cellular injury responsible for these shifts, as well as a growing understanding of a variety of complex metabolic and humoral responses to injury and shock. These advances and the growing focus on organ failure has to some extent shifted research emphasis from initial resuscitation to the pathophysiologic mechanisms of specific organ failure.

The post shock problem of multiple organ systems failure invovles lung, kidney, gut, stomach, liver and immune defense mechanism. In the past 3 years, in several separate experiments we have noted that after apparently successful hemodynamic resuscitation in primates blood flow to large and small intestine, stomach, spleen, kidney, and probably liver remain significantly reduced for at least 18 hours after resuscitation. Furthermore, blood voluem in spite of apparently effective resuscitation remains only 70 to 80% of baseline. The present study describes these changes in two experiments initially designed to evaluate two different hemodynamic parameters as indicators of effective resuscitation from hemorrhagic shock.

Materials and Methods:

Fourteen baboons (10-15 kg) were sedated with IV ketamine and anesthetized with IV pentothal. Subsequent sedation was provided with IV ketamine as needed. Animals were intubated and ventilated with a respirator. Blood gases were monitored during the procedure for adequacy of respiration.

Through a left thoracotomy a flow probe was placed around the pulmonary artery and pressure lines placed in the right atrium, left atrium and femoral artery. Studies of visceral blood flow were carried out with radioactive microspheres.

Hemorrhagic shock was inducjd by rapid exsanguination to a mean arterial pressure (MAP) of 60 mmHg for one hour followed by further hemorrhage to 40 mmHg for an additional hour. One group of animals (I - 5 animals) was resuscitated with shed blood and Ringer's lactate to restore MAP to baseline ±10% and maintained with additional Ringer's solution as needed for the next 20 hours. The other group (II - 8 animals) was resuscitated until LAP was baseline ±10% and maintained with Ringer's for 18 hours.

In both groups, hemodynamic data was monitored continuously and injection of radioactive microspheres (Ce^{141}, Cr^{51}, Sr^{85}, Sc^{46}) performed at baseline, following 2 hours of shock, 2 hours after resuscitation and 18 hours after resuscitation whereupon the experiment was terminated.

Blood volume determinations (Evan's blue) were made at baseline and at 18 hours in both groups. Recordings of intake and output were carefully maintained.

Blood flow determinations with isotope dilution were made from a method based on the principle that microspheres injected into the left atrium distribute systemically in proportion to blood flow to various parts of the body and are trapped in the precapillary arterioles in their first passage through the circulation. The number of particles in any region of the body, reflected by the radioactivity, is proportional to the flow to that region.

After sacrifice the organs to be studied are excised, rinsed in saline and put into formalin for 12 hours. Subsequently, representative wedge-shaped slices of each organ from a peripheral, intermediate and central position 5 mm x 5 mm are taken and placed into glass test tubes.

Spheres are suspended in 10 cm^3 of 10% dextran with one drop of Tween 80 to minimize clumping. They are labeled with gamma emitting nuclides. Four microsphere tags are utilized: ^{85}Sr, ^{51}Cr, ^{141}Ce and ^{46}Sc. Dosage administered is calculated for each animal based on the size of the animal and the predetermined activity which we determine for the microspheres. We require activities in excess of 50 cpm per tissue sample (sample at 0.5 to 1.0 gm). Spheres are

15±5 μm in size. In general, 2 to 2.5 million microspheres are suspended in 5 ml saline and injected over 10 seconds into the left atrium 20 to 30 hours after ligation. Before injection the vial containing microspheres is vigorously agitated on a mechanical mixer for 10 minutes and then placed into an ultrasonic bath for another 2 minutes to avoid clumping of the spheres. Two reference blood samples are withdrawn simultaneously from stiff-walled 5 Fr catheters in the brachial or carotid and a femoral artery with a Harvard withdrawal pump at a rate of 4 ml/min for 2 minutes beginning 15 seconds before injection of the microspheres. The catheters are tied into the arteries about 1 mm apart from the tip so that trapping of the microspheres between the catheter and the vessel wall can be avoided. The radioactivity of these samples is then determined in a Beckman scintillation counter.

The method implies that the ratio of the flow to (Q_k) and (C_k) in one organ (=reference samples) will be the same in all organs, therefore, the flow (Q_u) to any other organ can be calculated after its radioactivity (C_u):$Q_u=C_k \times C_u$ is measured. The relation of reference flow to reference radioactivity is averaged over the two samples.

Samples are counted in their appropriate windows (i.e., ^{85}Sr at a window of 470 to 570 KEV). Counts are then included in a series of simultaneous equations contained in our computer which returns values in actual counts and also converts these to blood flow based on reference samples as outlined above.

Results:

In Group I animals MAP, LAP, CVP and CO dropped during hemorrhage and returned to near baseline levels after resuscitation, MAP remained slightly below baseline, LAP and CVP measurements were not significantly reduced during resuscitation, although CO was somewhat lower 8 and 18 hours post resuscitation (Figs. 1a and 1b).

Blood flow to heart and brain were depressed during shock, although better maintained than other organs, and were normal throughout resuscitation (Figs. 2a-e). Kidney, spleen, gut and lung showed severely reduced blood flow during shock which returned to baseline or above 2 hours post resuscitation. However, at 18 hours all were significantly below baseline ($p<0.05$). Blood volume was also significnatly reduced to 72% of baseline (Table I).

Group II animals showed a drop in FAP, CVP, LAP and CO with shock and all values were restored to near baseline and maintained there for 18 hours post resuscitation (Figs. 3a-d). Attempts at maintaining LAP at absolute baseline were successful for the group at large but not in individual animals and $\overline{LAP}$ for the group was frequently slightly below baseline (Fig. 3c). Blood flow to heart and brain were not significantly depressed during shock but were elevated at 2 hours and back to normal by 18 hours (Fig. 4). Intestine, spleen, kidney and lung were significantly reduced during shock, returned to normal or above normal 2 hours post resuscitation and were below baseline at 18 hours, although the reduction was only significant in intestine and spleen ($p<0.05$).

Intake and output (Fig. 5) are shown for the 20 hours of the experiment as are blood volumes. Attempts to maintain LAP at baseline in each animal resulted in massive IV volumes which were largely excreted in the urine, did not increase LAP above baseline and did not alter the characteristic reduction in blood volume remaining 77% of baseline ($p<0.05$) at 18 hours (Table I).

Discussion:

Three observations from the present study deserve further discussion and emphasis: 1) that blood volume remains depressed 18 hours after hemodynamic resuscitation from hemorrhagic shock, 2) that abdominal visceral blood flow is reduced significantly for at least 18 hours after resuscitation from hemorrhagic shock, and 3) that it is not possible to use LAP as a hemodynamic guideline for resuscitation.

Blood volume remained significantly reduced after "successful" resuscitation in both groups of animals. Similar observations were made by Simmons, et. al.[3] in combat casualties, who summarized their data stating, "The results suggest that a severe degree of shock is associated with a contracted vascular space which is not expanded during resuscitation even by large quantities of blood and crystalloid solution".

Blood flow to abdominal viscera, most notably the gut and spleen but including the kidney and lung and probably liver as well, occurred at 18 hours in both groups of animals though both by normally monitored hemodynamic criteria were well resuscitated.

This is particularly interesting in light of the post shock multisystems organ failure problems which are the major late serious complications after apparently successful resuscitation from shock in humans. These include post traumatic pulmonary insufficiency (lung), renal failure (kidney), sepsis and immunosuppression (reticuloendothelial system - liver and spleen), stress ulcer (gut) and hepatic failure (liver).[4]

George and co-workers[5] have shown significant evidence of tissue injury persisting for several days in abdominal viscera after an episode of shock. Similarly, McArdle and co-workers[6] have shown that intestinal levels of ATP and cyclic AMP did not recover after resuscitation. Similarly, Loegering[7] has demonstrated depression of the reticuloendothelial system in hypovolemic shock. It seems likely that persistent reduced visceral blood flow would compound the ischemic injury to both liver, stomach and kidney as well. Our reference to reduced hepatic blood flow is primarily by inference since the measured blood flow represented only systemic (i.e., hepatic arterial) flow. However, since most of hepatic blood flow is from the portal circulation and the measured flows in this system are all reduced it seems reasonable to assume that liver blood flow is reduced as well.

The mechanism for this persistent reduction in visceral blood flow is unknown. We have speculated that it represents persistence of a teleologically important response to shock which redistributes blood flow but is not shut off by volume replacement alone.

Finally, some mention of the observation that in spite of massive volume replacement, volume could not be expanded. Not only was blood volume not expanded in Group II at 18 hours but massive fluid replacement was accomplished by concomitant massive urinary excretion without a significant rise in LAP. It is evident that LAP is not a useful index of resuscitation in shock. Trunkey and co-workers[8] have previously stated that CVP and urine output are the best clinical parameters of resuscitation from shock in baboons and Calvin and co-workers[9] have noted that pulmonary wedge pressure is a poor predictor of volume status after resuscitation from shock in man.

Virtually all three of the above discussed phenomenon suggest persistence of a compensatory mechanism for the condition of hypovolemic shock beyond the time it is needed and even to the point that it may become deleterious. This mechanism is unknown and should be the topic for further investigation.

Bibliography

1. Blalock A: Principles of Surgical Care, Shock and Other Problems. C.V. Mosby Co., St. Louis, MO, 1940.
2. Wiggers CJ: Present status of shock problem. Physiol Rev 22:74, 1942.
3. Simmons RL, Heisterkamp CA III, Mosely RV, Doty DD: Post resuscitation blood volumes in combat casualties. Surg Gynecol Obstet 128:1193, 1969.
4. Shires GT, Canizaro PC, Carrico CJ: Shock. In Schwartz, et al, Principles of Surgery, 3rd Ed., McGraw-Hill, New York, 1979, pp 135-184.
5. George BC, Ryan NT, Ullnick WC, Egdahl RH: Persisting structural abnormalities in liver, kidney and muscle tissues following hemorrhagic shock. Arch Surg 113:289, 1978.
6. McArdle AH, Chiu CJ, Hinchey EJ: Cyclic AMP response to epinephrine and shock. Arch Surg 110:316, 1975.
7. Loegering DJ: Humoral factor depletion and reticulo-endothelial depression during hemorrhagic shock. Am J Physiol 232:H283, 1977.
8. Trunkey D, Holcroft J, Carpenter MA: Monitoring resuscitation of primates from hemorrhagic and septic shock. JACEP 5:249, 1976.
9. Calvin JE, Driedger AA, Sibbald WJ: Does the pulmonary capillary wedge pressure predict left ventricular preload in critically ill patients? Crit Care Med 9:437, 1981.

Table I

	IV Fluids Given Over 20 Hrs (ml)	Blood Volume at 20 Hrs (Percent of Baseline)
Group I	2015	72 ± 9.88
Group II	7200	78 ± 10.6

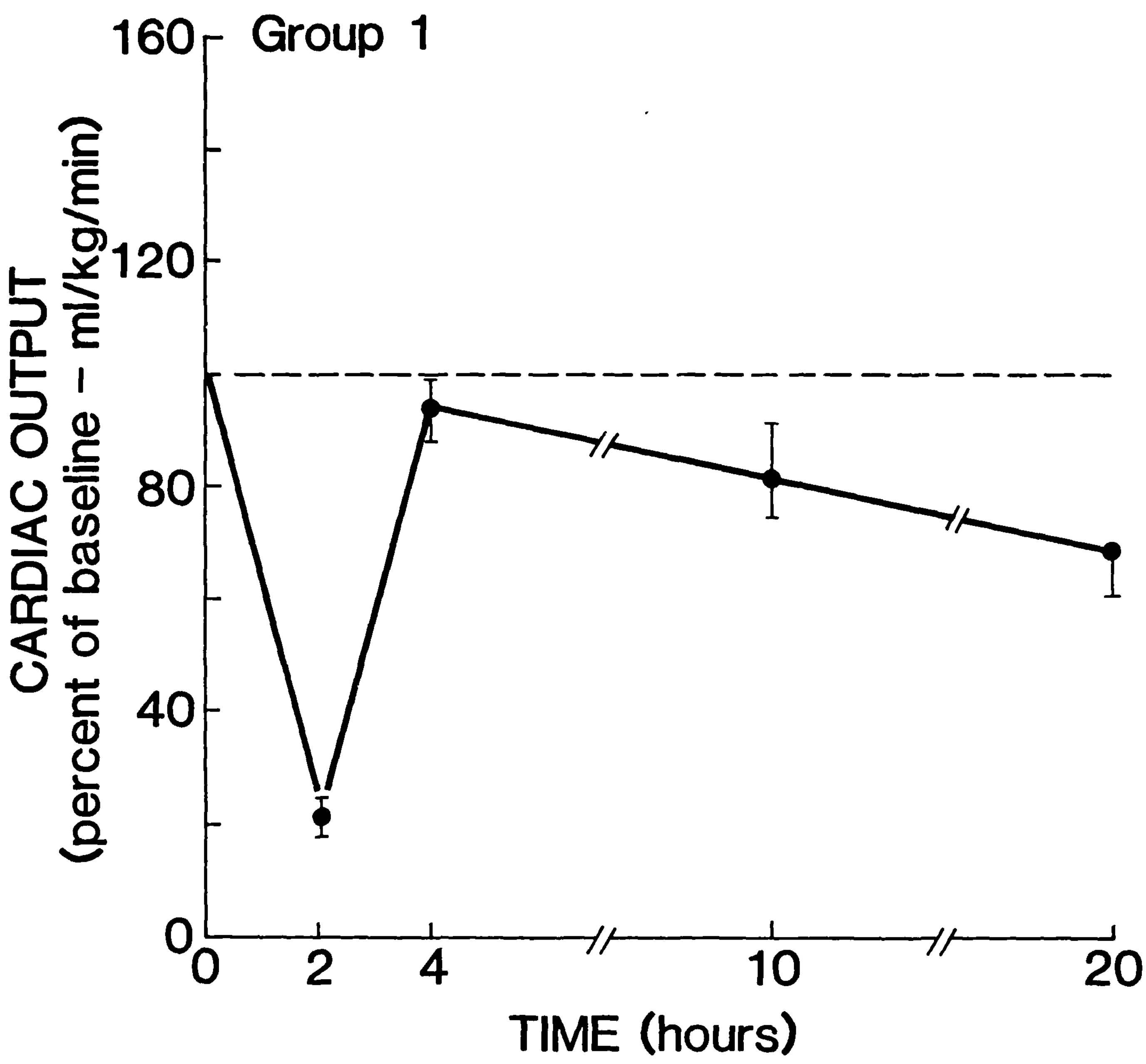

Group 1
CARDIAC OUTPUT
(percent of baseline - ml/kg/min)
160
120
80
40
0
0
2
4
10
20
TIME (hours)

Fig. 1a

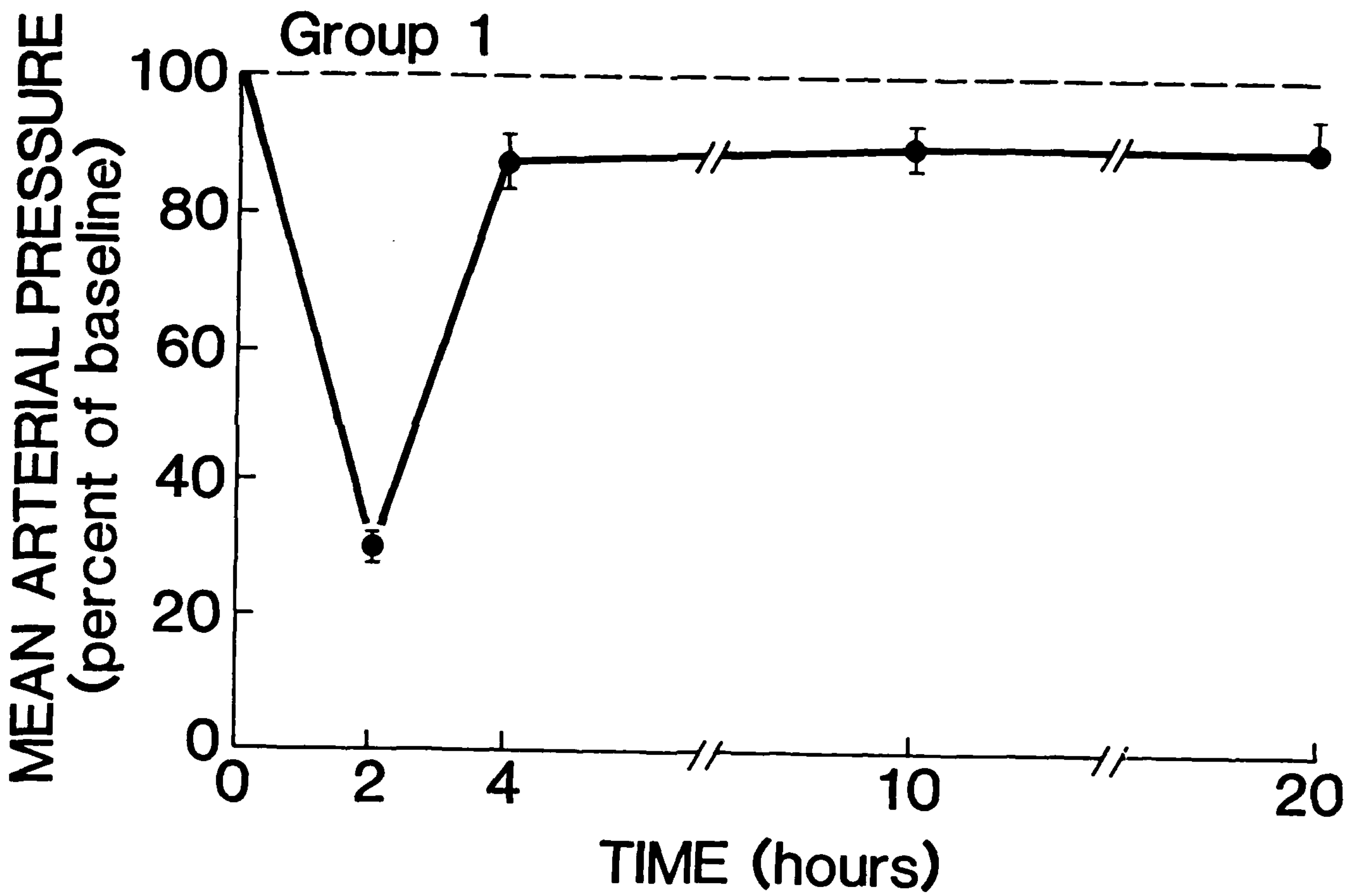

Group 1
MEAN ARTERIAL PRESSURE
(percent of baseline)
100
80
60
40
20
0
0
2
4
10
20
TIME (hours)

Fig. 1b

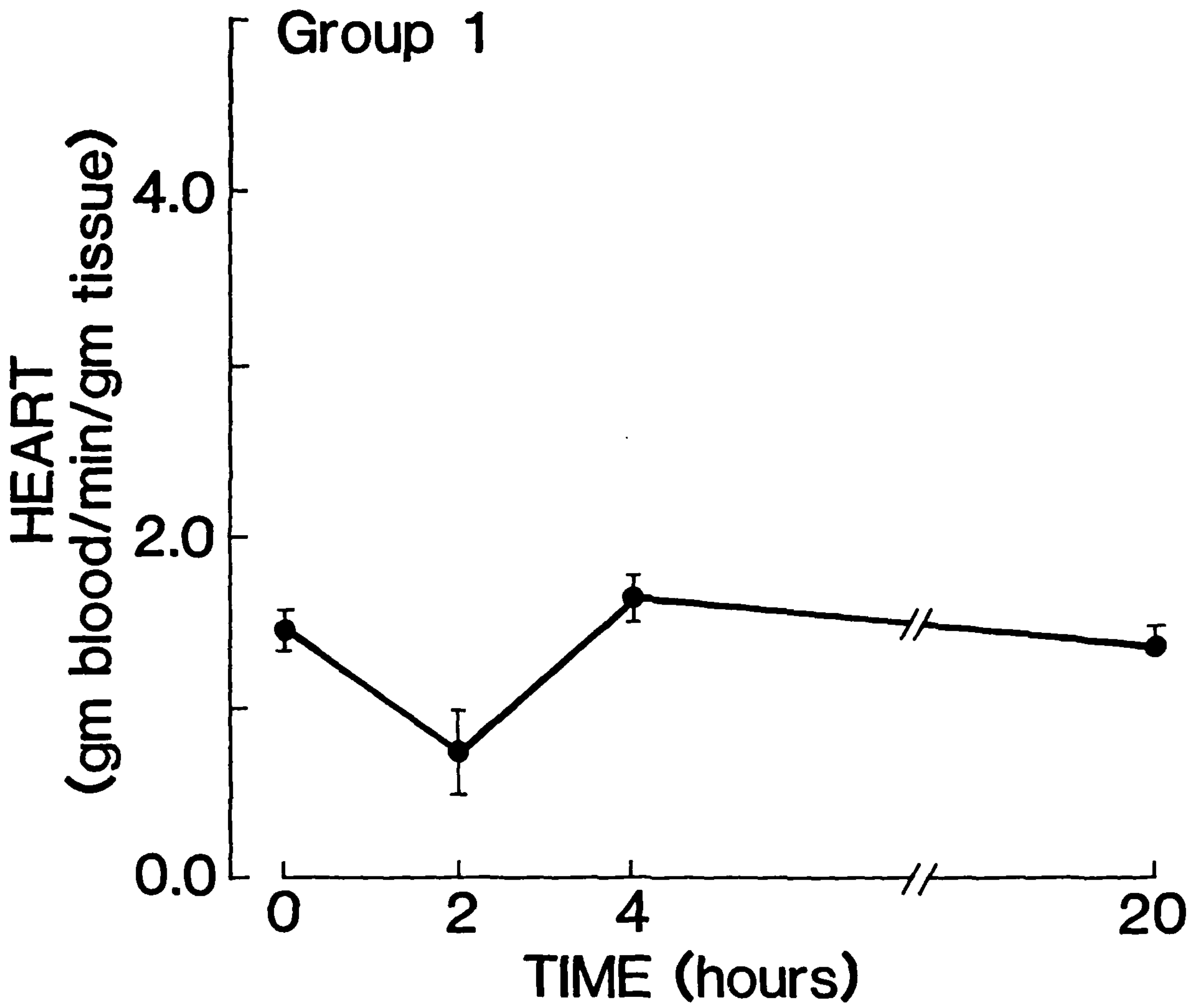


Fig. 2a

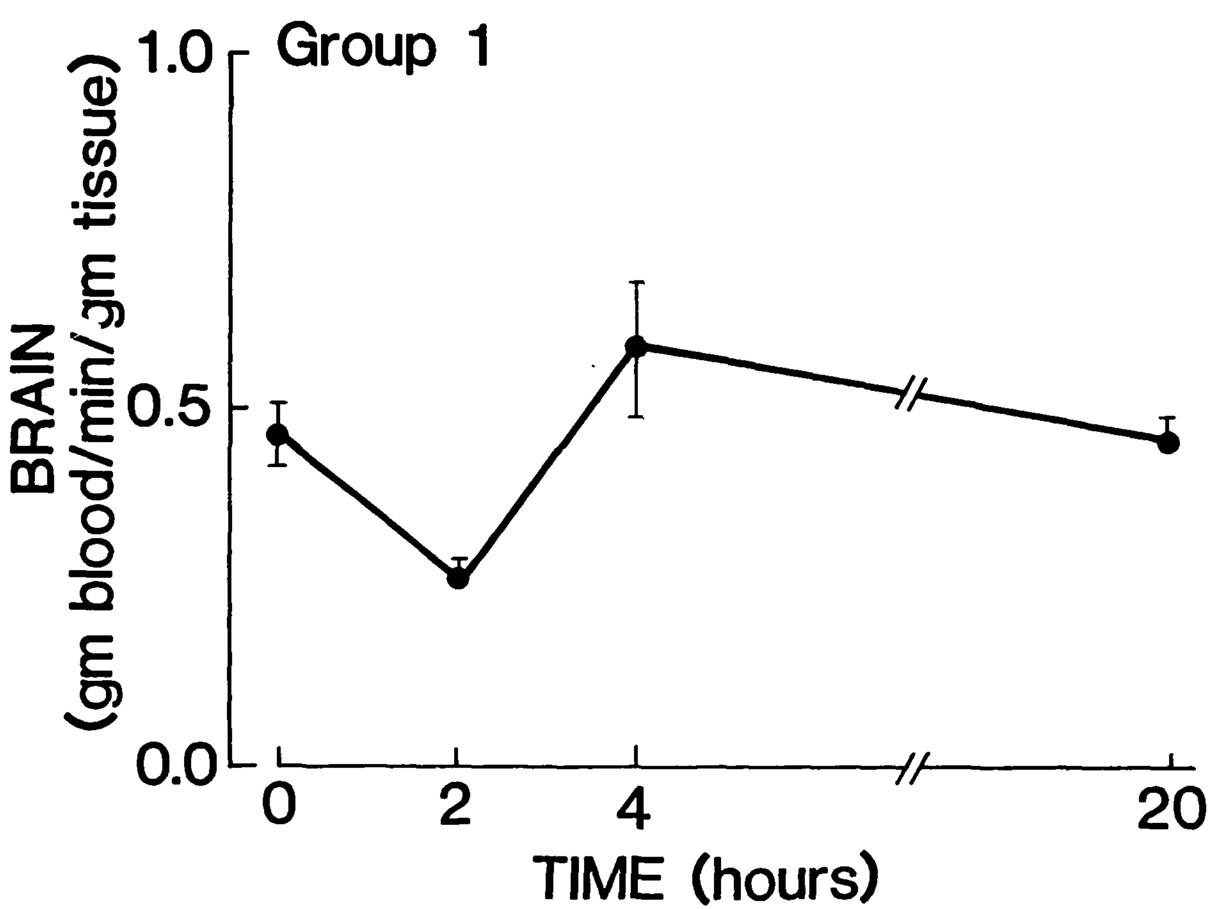


Fig. 2b

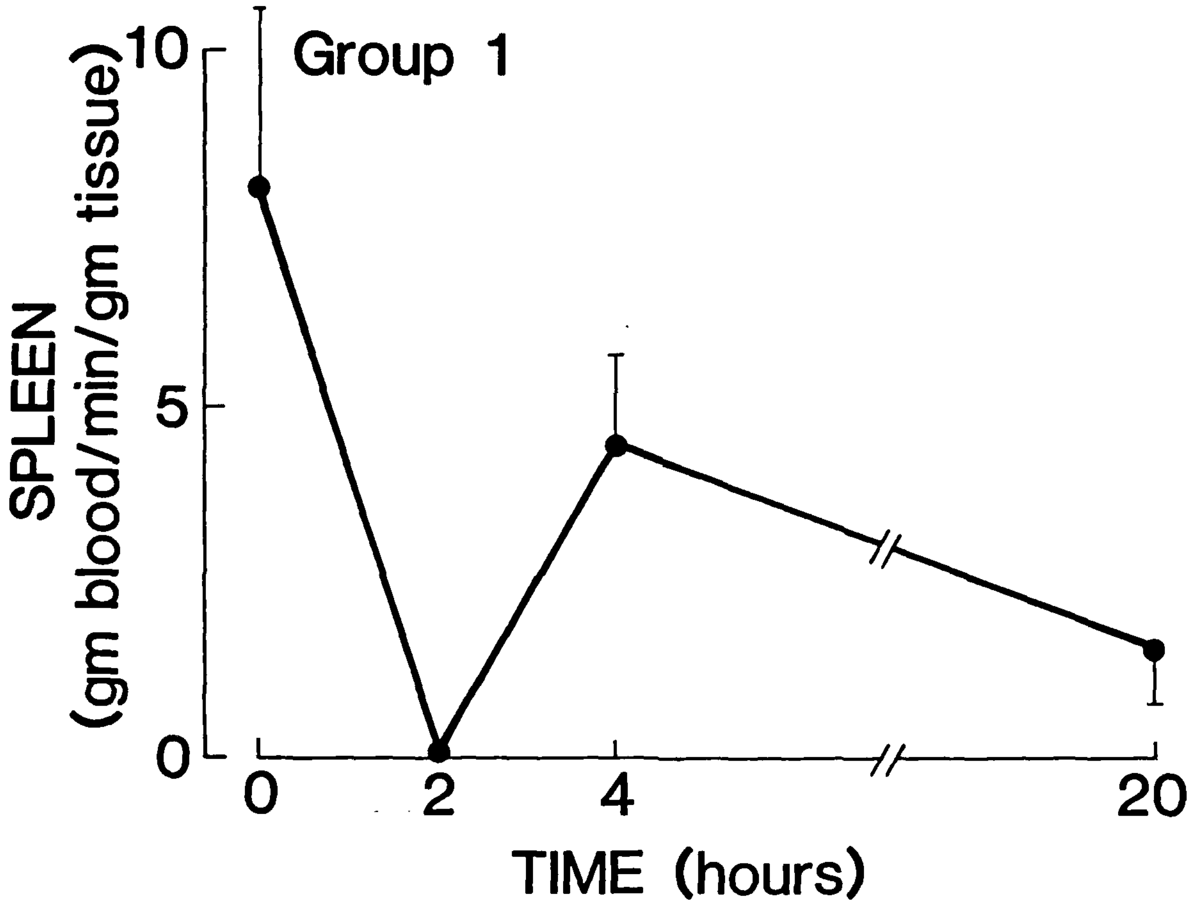


Fig. 2c

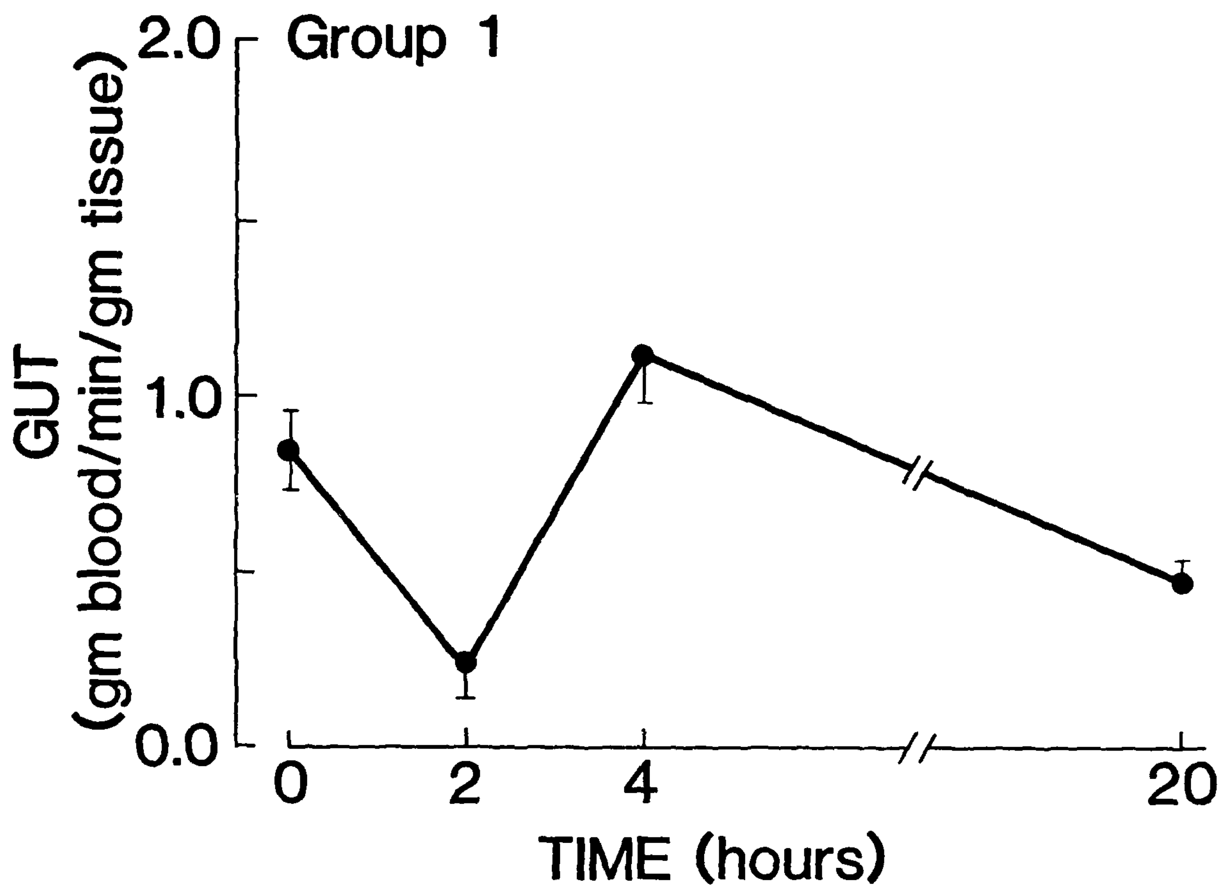


Fig. 2d

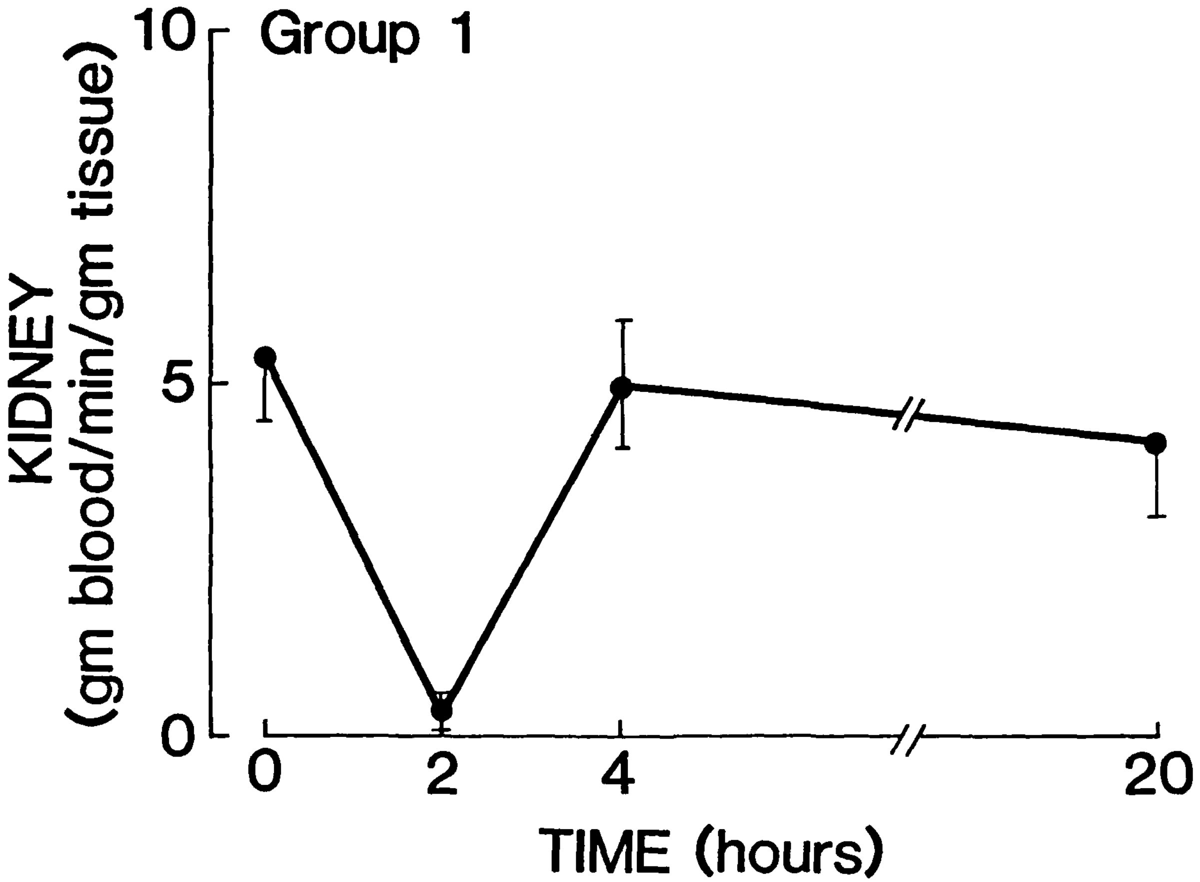
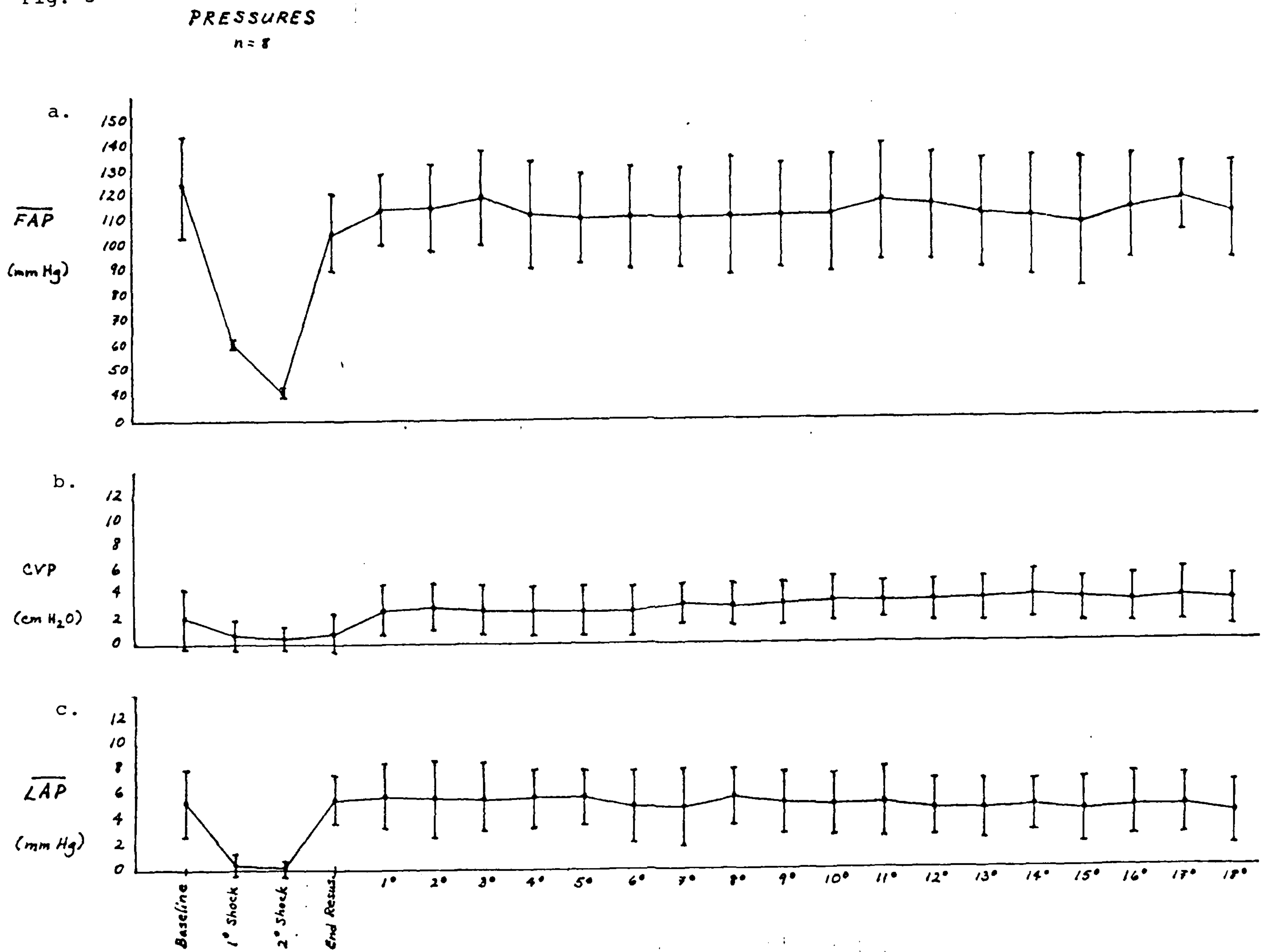


Fig. 2e

Fig. 3



PRESSURES
n = 8
a.
$\overline{FAP}$
(mm Hg)
150
140
130
120
110
100
90
80
70
60
50
40
0
b.
CVP
(cm H_2O)
12
10
8
6
4
2
0
c.
$\overline{LAP}$
(mm Hg)
12
10
8
6
4
2
0
Baseline
1° Shock
2° Shock
End Resus.
1°
2°
3°
4°
5°
6°
7°
8°
9°
10°
11°
12°
13°
14°
15°
16°
17°
18°

Fig. 3d

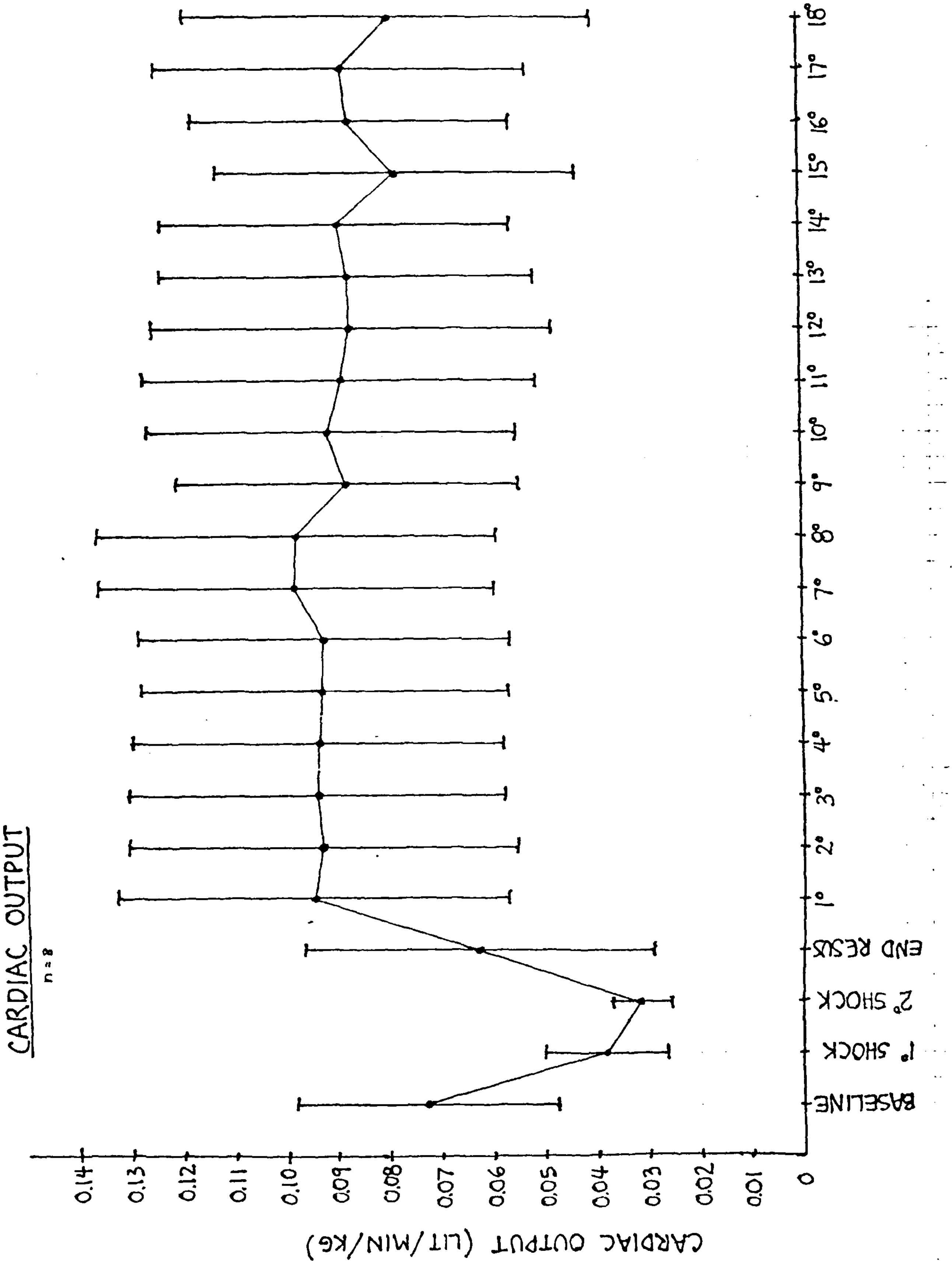

CARDIAC OUTPUT
n=8
CARDIAC OUTPUT (LIT/MIN/KG)
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
0.12
0.13
0.14
BASELINE
1° SHOCK
2° SHOCK
END RESUS
1°
2°
3°
4°
5°
6°
7°
8°
9°
10°
11°
12°
13°
14°
15°
16°
17°
18°

Fig. 4

MICMATX.DAT   Tissue Perfusion:
n = 8

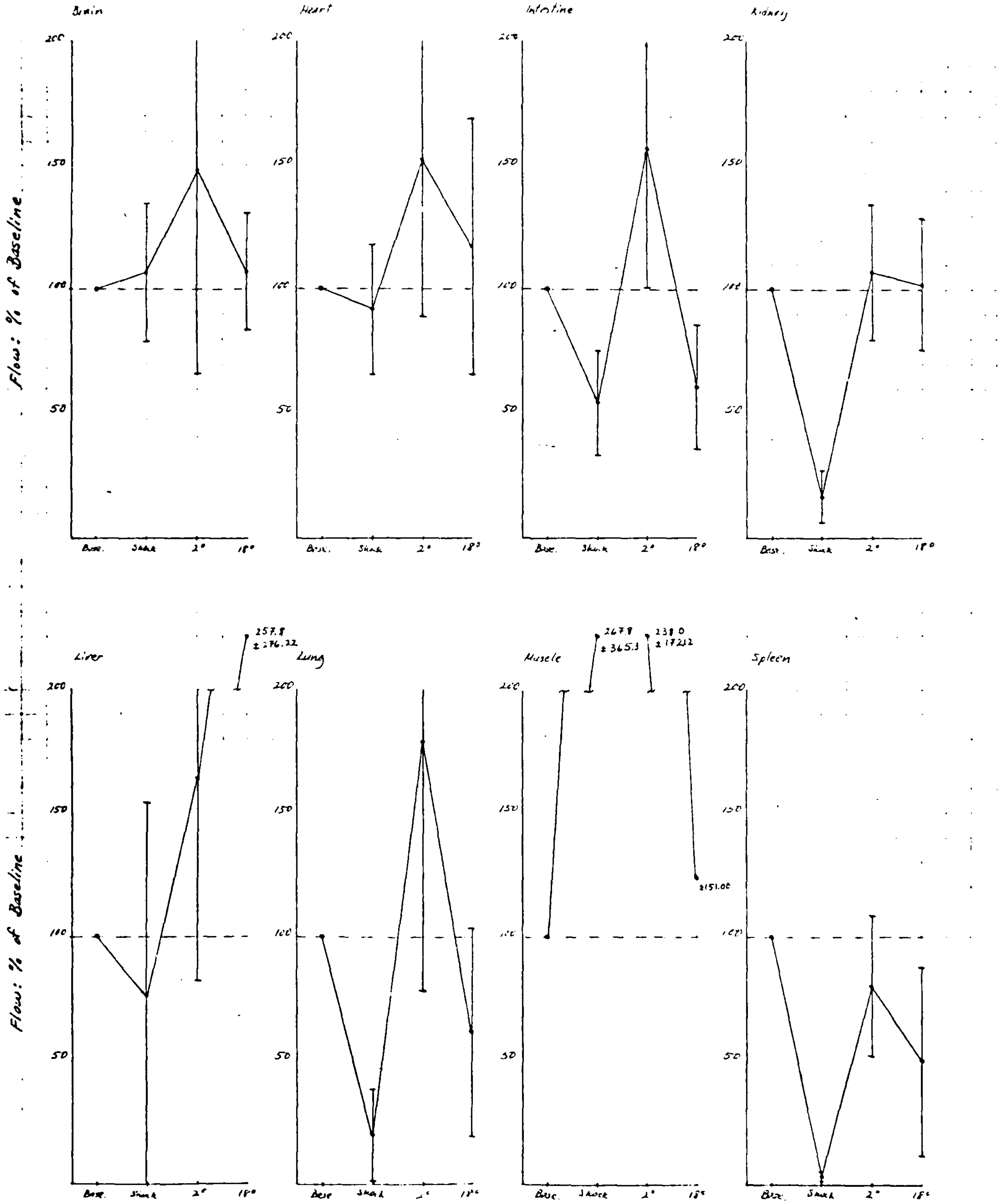
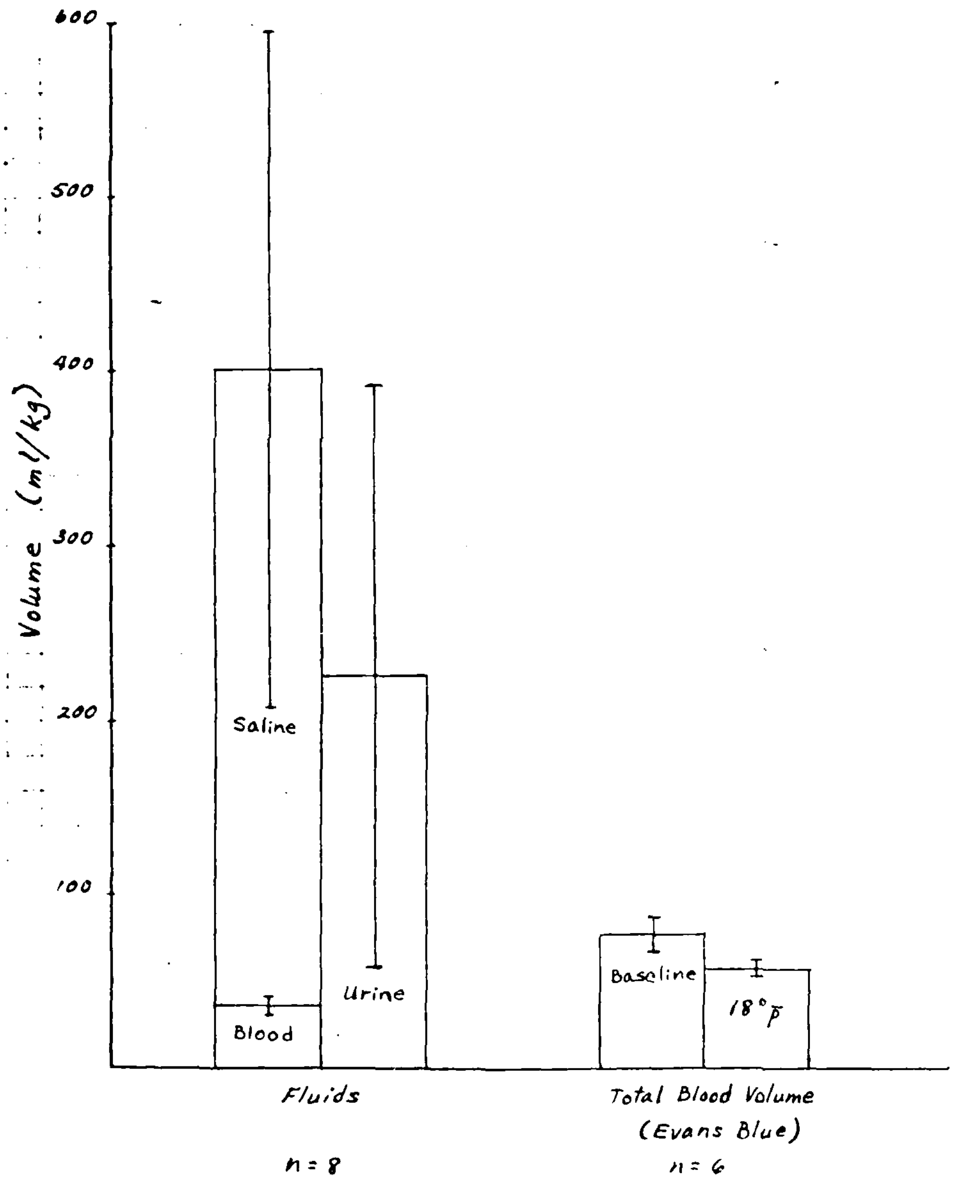


Fig. 5

Fluid Dynamics - Group 2



Angiotensin II and Prostaglandins in Hemorrhagic Shock

Hemorrhagic shock is a complex interaction of physiological and biochemical processes. A not infrequent reality confronting the su.geon who sees trauma with some regularity is the patient who succumbs to multiple organ failure, coagulopathy, and sepsis some days to weeks following an apparently successful resuscitation from hemorrhagic shock. Despite hemodynamic resuscitation with blood and crystalloid, these patients pursue an inexorable downhill course finally yielding to disease processes seemingly unrelated to the initial insult. It is well known that in hemorrhagic shock, blood flow to the brain and heart is relatively well preserved at the expense of perfusion to the abdominal viscera and skeletal muscle. Furthermore, this redistribution persists as long as 18 hours following apparently adequate hemodynamic resuscitation. The neural and humoral regulation of this maldistribution is not well understood and the question of local modulators of the vasculature has been the subject of intensive investigation.

Recently, considerable attention has been directed to the effects of prostaglandins on the vasculature. Prostacyclin, a naturally occurring derivative of arachidonic acid metabolism is a vasodilator and an inhibitor of platelet aggregation. Thromboxane A_2 is produced by platelets and has the opposing actions of vasoconstriction and promoting platelet aggregation. Angiotensin II production is dependent on the amount of renin released from the kidney and one mechanism for its release is prostaglandin mediated.

SLIDE (Fig. 1)

The aim of this research was to determine whether prostaglandins were involved in hemorrhagic shock. Specifically, we wanted to see if thromboxane was a mediator of persistent vasoconstriction. In addition, we wanted to establish the pattern of renin release in a non-resuscitated shock model. Finally, we wanted to see the effect of prostaglandin inhibition on angiotensin, thromboxane, and survival.

SLIDE (Fig. 2)

Twenty New Zealand white rabbits were studied in two groups. The ten experimental animals were given oral children's chewable aspirin at about 30 mg/kg four times daily for two days prior to the experiment. They were allowed free access to water, but all animals were fasted for the last 12 hours. On the morning of the procedure, they were sedated with ketamine and anesthetized with pentobarbital. Subcutaneous infiltration with Xylocaine was done to minimize pain and the need for prolonged anesthesia. Access to the femoral vessels was accomplished for exsanguination and the recording of hemodynamic data. Following tracheostomy and the institution of mechanical ventilation, a left anterior thoracotomy was performed to place a left atrial catheter. The chest was closed and evacuated. The animals were weaned from the respirator and light sedation was maintained by small intravenous infusions of pentobarbital. In no case was pentobarbital administered following the induction of shock.

They were allowed one hour for stabilization prior to the recording of baseline parameters. Blood volume was measured using the Evan's blue dye dilution technique. Radioactive microspheres were infused into the left atrium to calculate organ perfusion. Radioimmunoassays of $PGF_{1\alpha}$, TxB_2, and Angiotensin I generation were performed using the chemicals and protocol supplied by New England Nuclear.

Following the recording of baseline parameters and the initial infusion of radioactive microspheres, 35% of the measured blood volume was withdrawn over 10 minutes. Arterial blood was obtained for subsequent radioimmunoassays and immediately placed on ice. The plasma was separated in a refrigerated centrifuge and stored at -20°C. Shed blood was not returned as the animals were allowed to spontaneously resuscitate. Five percent dextrose in lactated Ringer's solution was infused at 10 ml/hr beginning at two hours. The animals were monitored for sixteen hours whereupon the experiment was terminated. The survivors were sacrificed and tissue was removed from all subjects to calculate organ perfusion by counting gamma emission.

SLIDE (Fig. 3)

The severity of the hemorrhage is reflected here. The mean arterial pressure was rapidly reduced to the mid 30's. The hypotension persisted despite a modest spontaneous recovery. No differences exist between the two groups except at 15 minutes when the aspirin treated group had a significantly higher pressure.

SLIDE (Fig. 4)

The effect of aspirin on platelets is seen by the reduction in aggregation in response to collagen. Aggregation to ADP, epinephrine, and serotonin was predictably similar.

SLIDE (Fig. 5)

There was an immediate decline in platelet count which persisted throughout the experiment. In both groups the response was similar.

SLIDE (Fig. 6)

In the control animals, this decline in platelet count was preceded by a dramatic rise in plasma thromboxane. Thromboxane peaked with 10 minutes and thereafter progressively declined to low levels.

SLIDE (Fig. 7)

In the aspirin treated group, the response was totally abolished.

SLIDE (Fig. 8)

Even though baseline concentrations of thromboxane differed by a power of 25, perfusion to brain, heart, lung, gut, kidney, liver, and muscle was similar.

SLIDE (Fig. 9)

Prostacyclin was consistently lower in the treated animals and subsequent samples did not differ from baseline in either group.

SLIDE (Fig. 10)

The baseline renin activities were identical. The one hour sample demonstrated the peak concentrations and at that time, the aspirin treated animals had lower levels. By four hours, the concentration of renin had returned to baseline values and it remained there for the duration of the experiment.

SLIDE (Fig. 11)

Survival was significantly reduced in those animals treated with aspirin. The difference in mortality occurred during the initial two hours. Thereafter, the slope of the survival curves were parallel.

SLIDE (Fig. 12)

The nature of this protocol allowed pretreatment to have minimal influence on the induction of shock and it also removed the arbitrary nature of resuscitation. This study clearly demonstrates the activity of platelets and thromboxane in the acute response to hemorrhage. Thromboxane peaks rapidly, but then quickly subsides to very low levels. In such a non-resuscitated model, presumably the same stimuli for the initial release would be operating throughout the experiment. If this is the case, then it is conceivable that the available pool of arachidonic acid becomes exhausted or that some feedback mechanism operates to inhibit production. The similarity in visceral perfusion despite significantly different concentrations of thromboxane suggest little physiologic in the regulation of vascular tone in this model. The reason for the reduced survival is unclear, and it may be unrelated to the vasoactive and platelet aggregating properties of thromboxane. It is obvious from this work, however, that thromboxane can not be important in the persistent vasoconstriction seen in shock.

In this model, renin levels do not remain elevated and, in fact, return to baseline by four hours even without restoration of arterial pressure or blood volume. This suggests a finite capacity to produce renin acutely. Whether this is a function of depleation of substrate, profound renal ischemia, or feedback inhibition is unclear. But the use of a converting enzyme inhibitor to alleviate visceral congestion and to restore perfusion does not seem to be indicated.

It is important to continue the investigation of the biochemical basis for hemorrhagic shock. But the pathophysiology is very complex and the results are often difficult to define. Survival is the gold standard. What effect aspirin has on prostaglandin metabolism to cause that difference merits further work and thromboxane may be only a small part of the answer.

Objectives

- Role of Thromboxane A_2 in hemorrhagic shock.
- Pattern of renin release in a non-resuscitated shock model.
- Effect of prostaglandin inhibition on Thromboxane A_2, Angiotensin II and survival.

Fig. 1

Materials and Methods

New Zealand white rabbits
- 10 Control
- 10 Experimental—Treated with oral aspirin (30 mg/kg) four times daily for two days.

Hemorrhage—35% of the blood volume over 10 minutes.

No resuscitation with shed blood.

Fig. 2

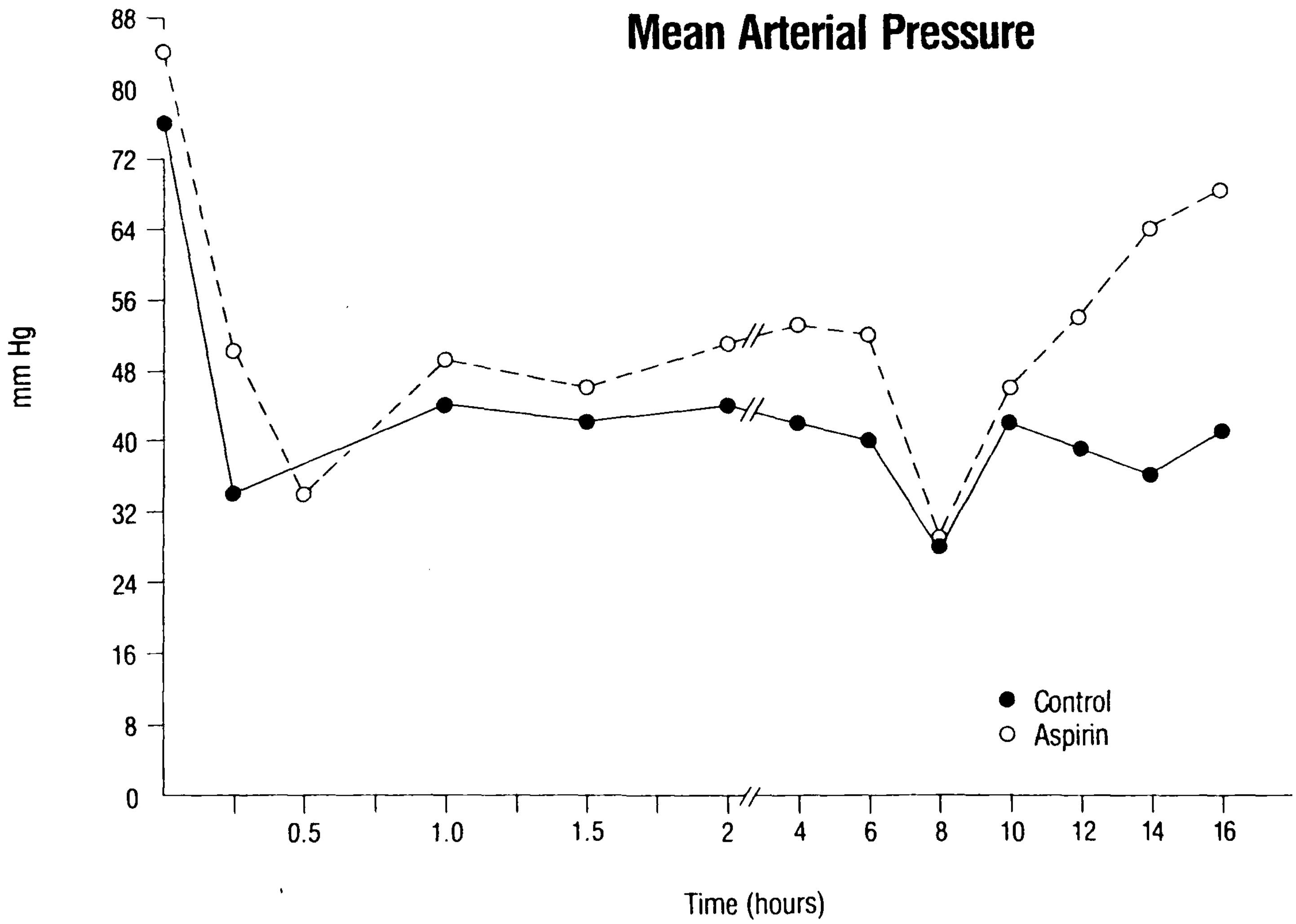

Mean Arterial Pressure
mm Hg
88
80
72
64
56
48
40
32
24
16
8
0
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)
Control
Aspirin

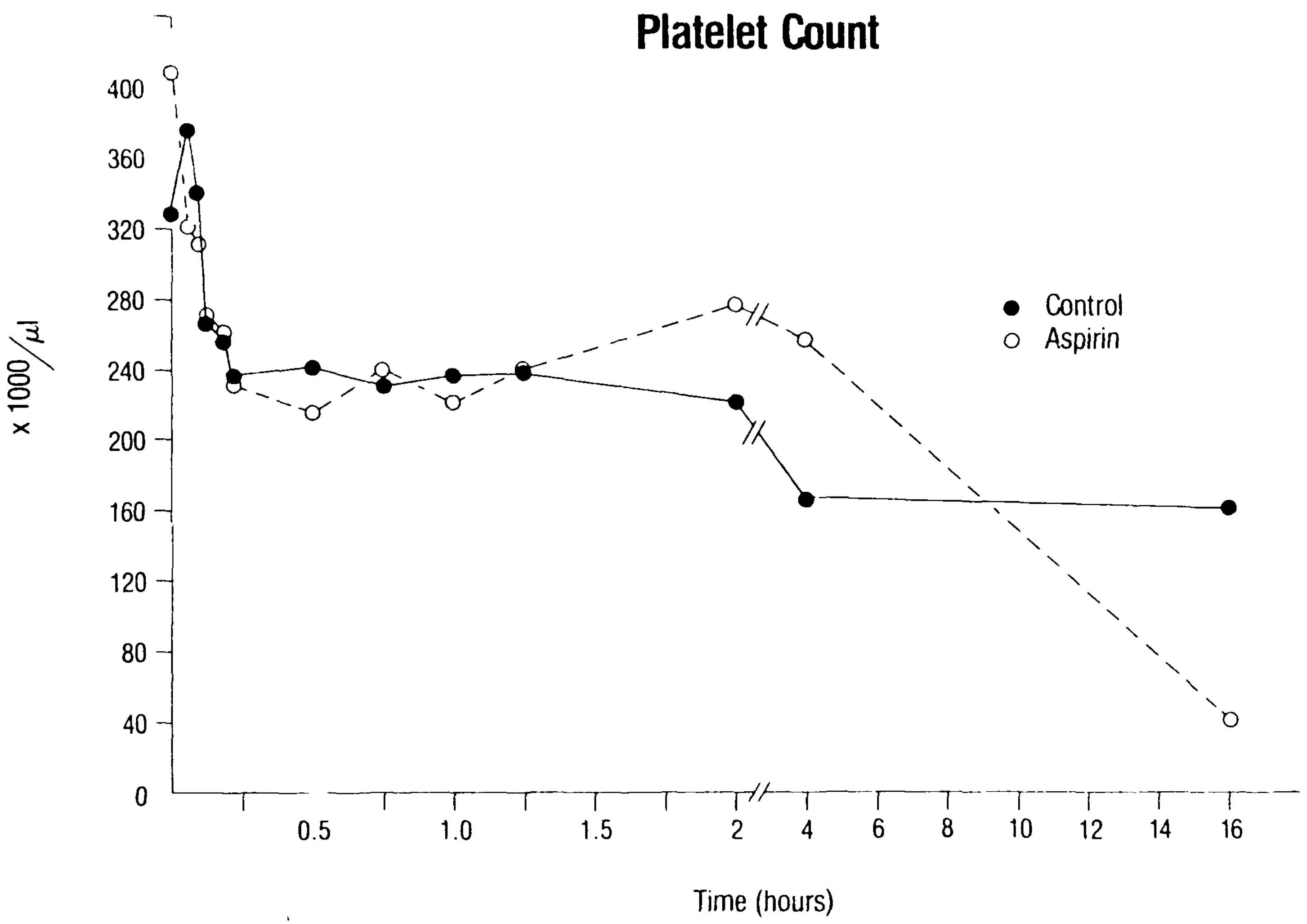
Fig. 3

Platelet Aggregation at Baseline

	ADP	Collagen*	Epinephrine	Serotonin
Control	66.72 ± 6.76	54.27 ± 10.51	10.60 ± 3.22	11.00 ± 2.85
ASA	66.55 ± 3.01	19.44 ± 10.66	16.50 ± 7.28	7.88 ± 4.31

*$p < 0.05$

Fig. 4



Platelet Count
x 1000/µl
400
360
320
280
240
200
160
120
80
40
0
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)
Control
Aspirin

Fig. 5

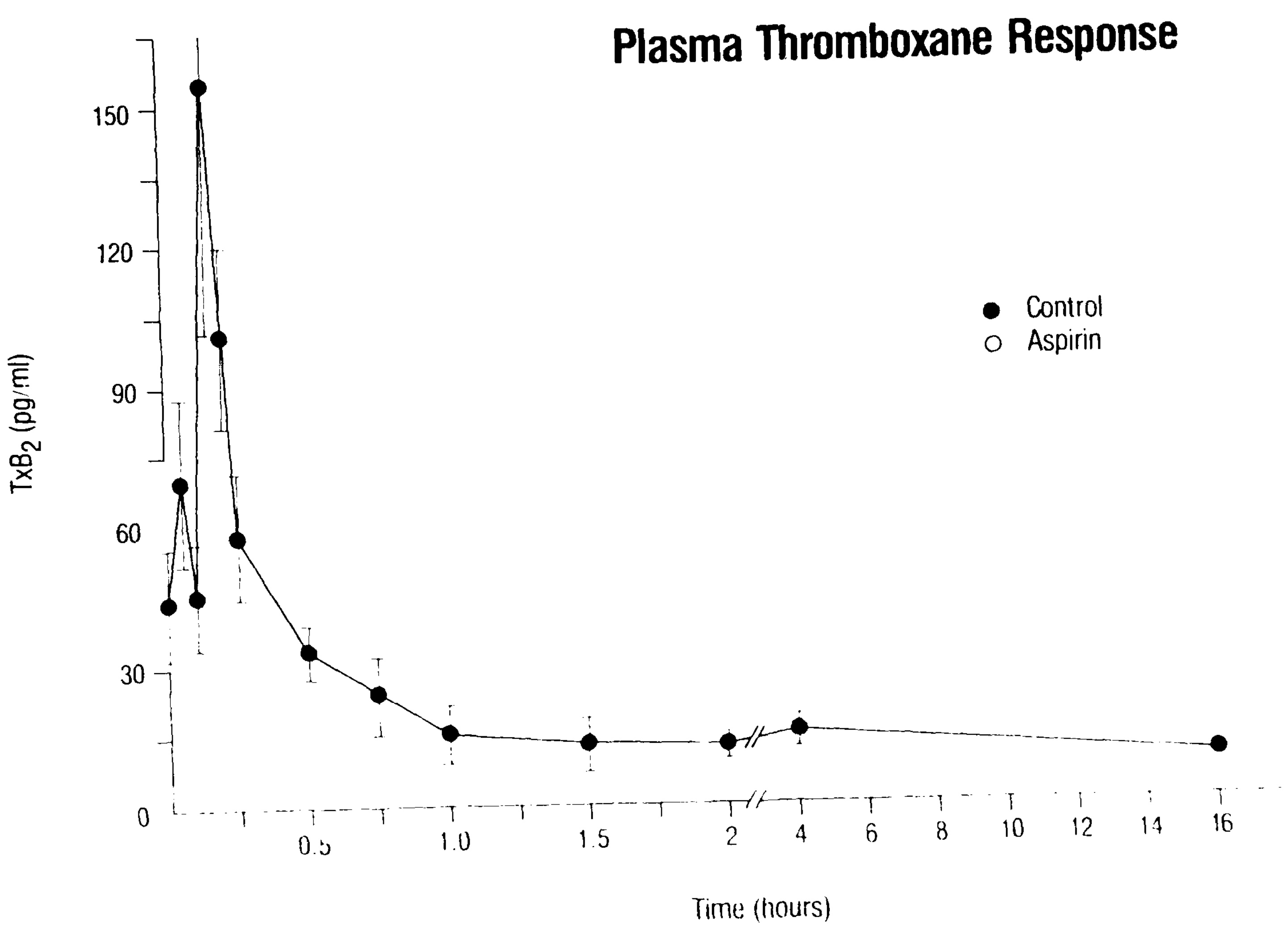

Plasma Thromboxane Response
Control
Aspirin
TxB_2 (pg/ml)
150
120
90
60
30
0
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)

Fig. 6

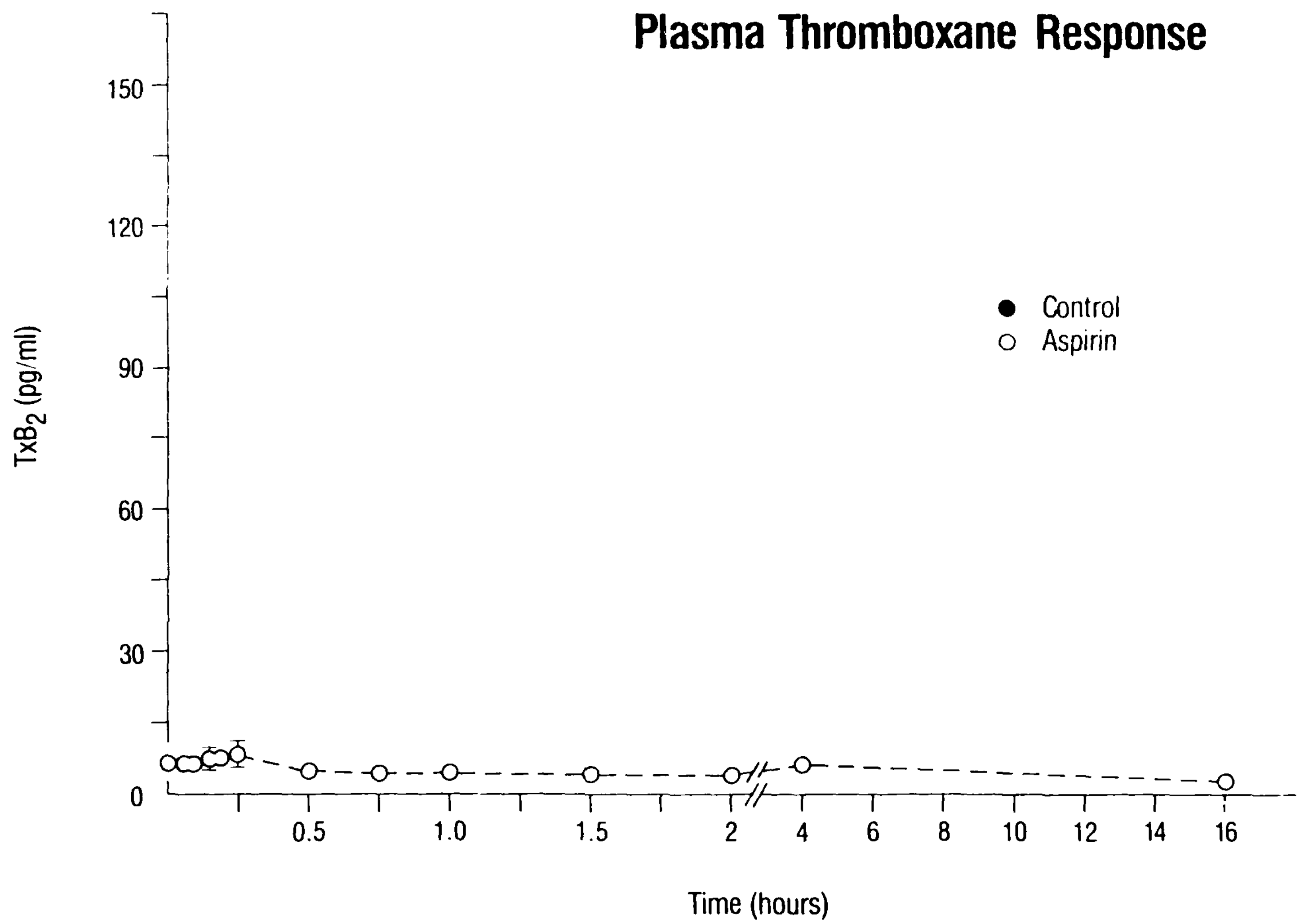

Plasma Thromboxane Response
Control
Aspirin
TxB2 (pg/ml)
150
120
90
60
30
0
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)

Fig. 7

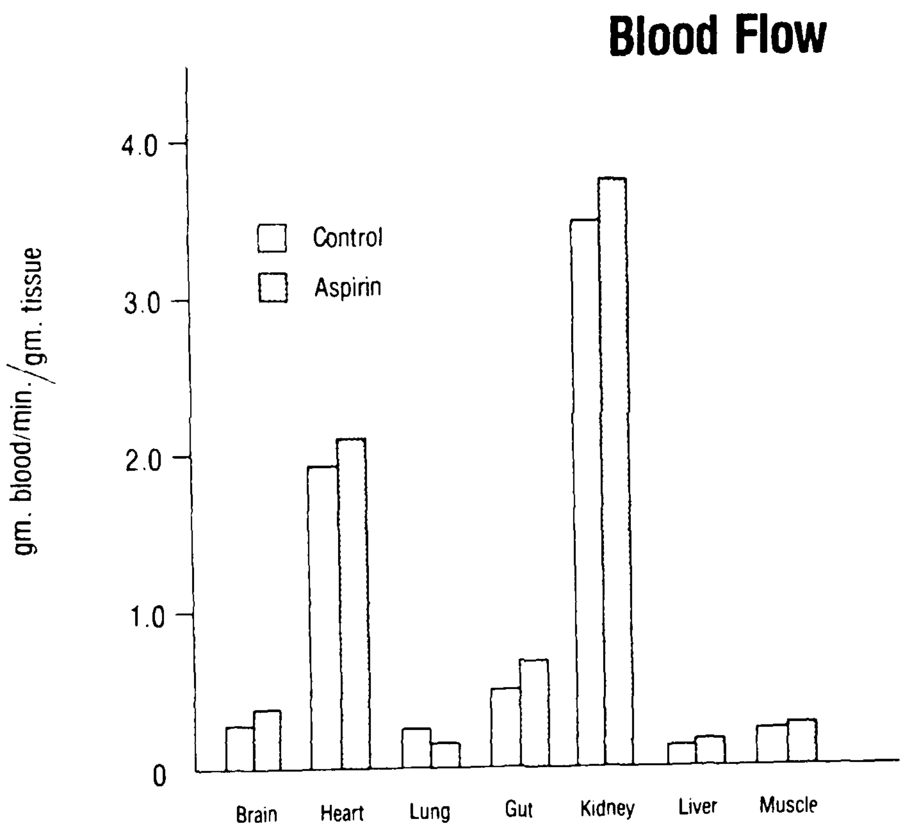

Blood Flow
4.0
3.0
2.0
1.0
0
gm. blood/min./gm. tissue
Control
Aspirin
Brain
Heart
Lung
Gut
Kidney
Liver
Muscle

Fig. 8

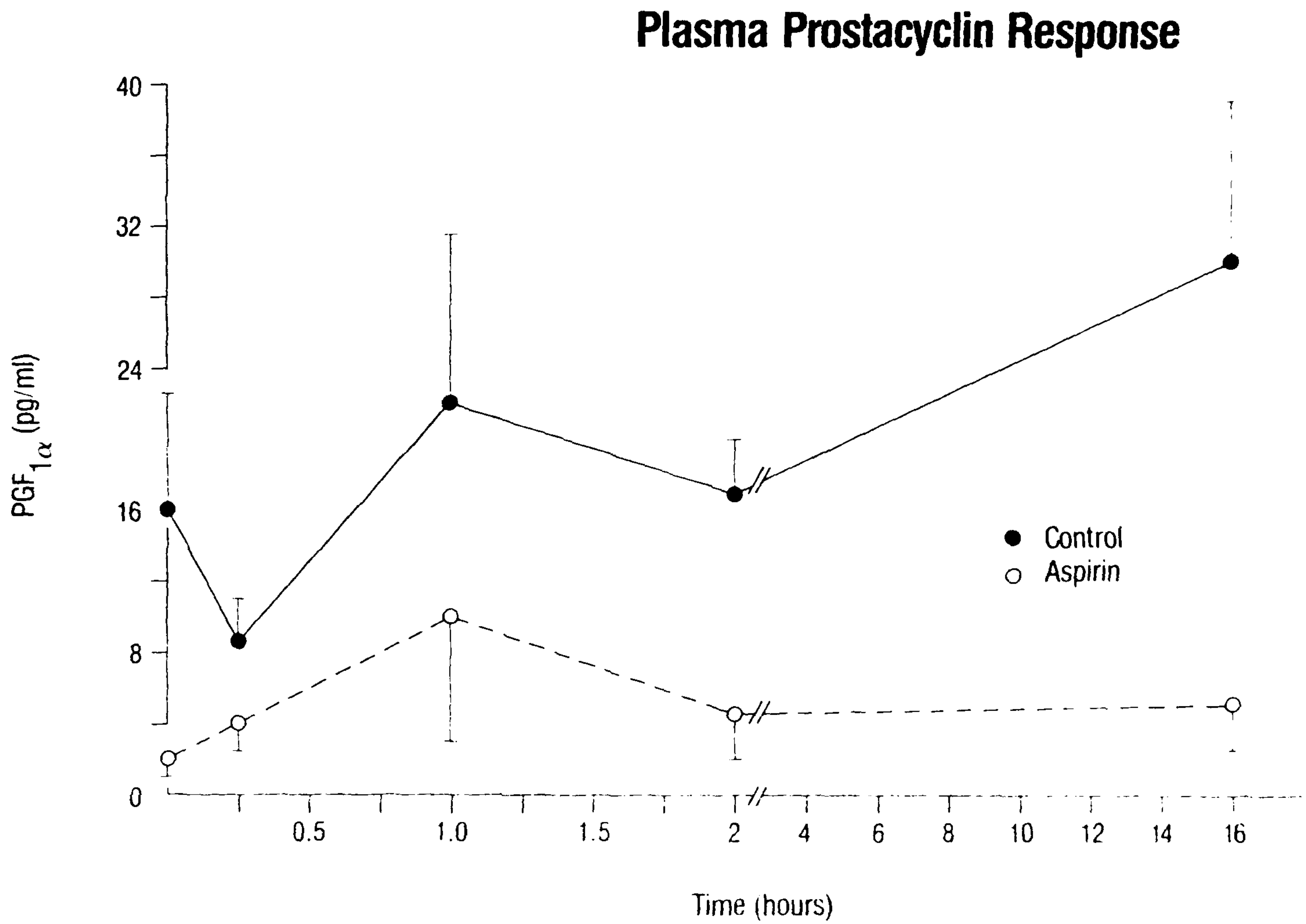
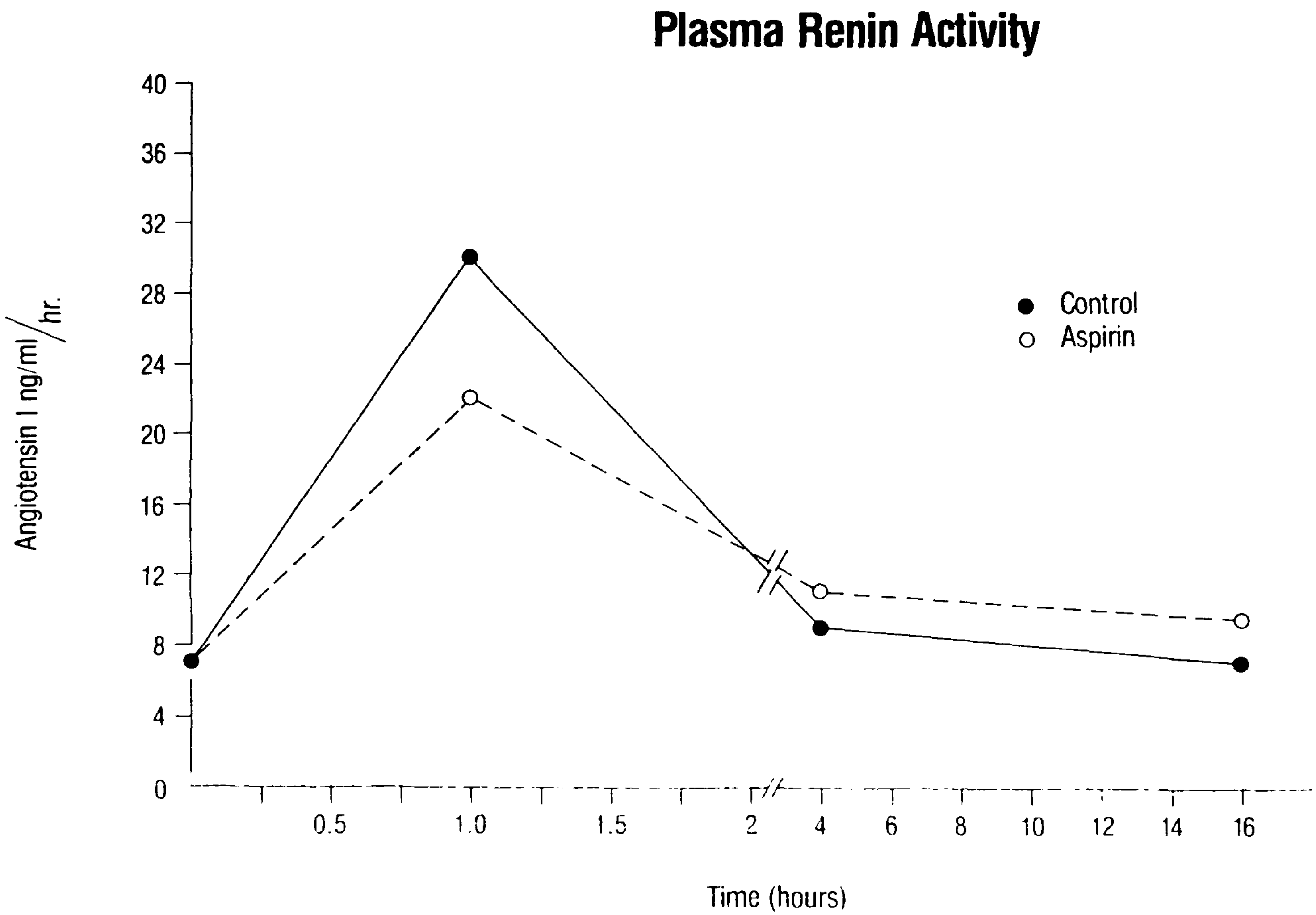

Plasma Prostacyclin Response
40
32
24
16
8
0
PGF1α (pg/ml)
Control
Aspirin
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)

Fig. 9



Plasma Renin Activity
Angiotensin I ng/ml/hr.
40
36
32
28
24
20
16
12
8
4
0
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)
Control
Aspirin

Fig. 10

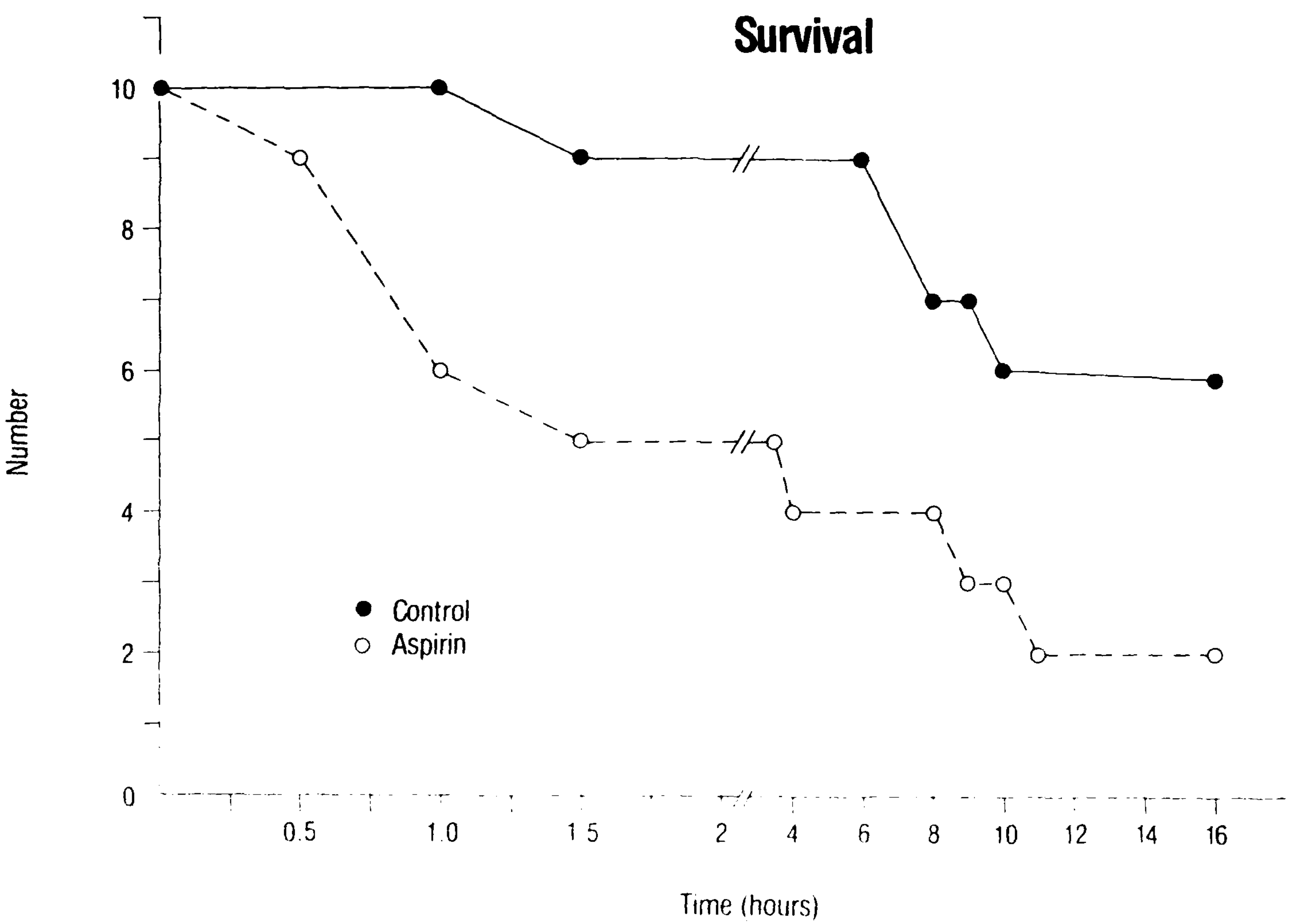

Survival
Number
10
8
6
4
2
0
Control
Aspirin
0.5
1.0
1.5
2
4
6
8
10
12
14
16
Time (hours)

Fig. 11

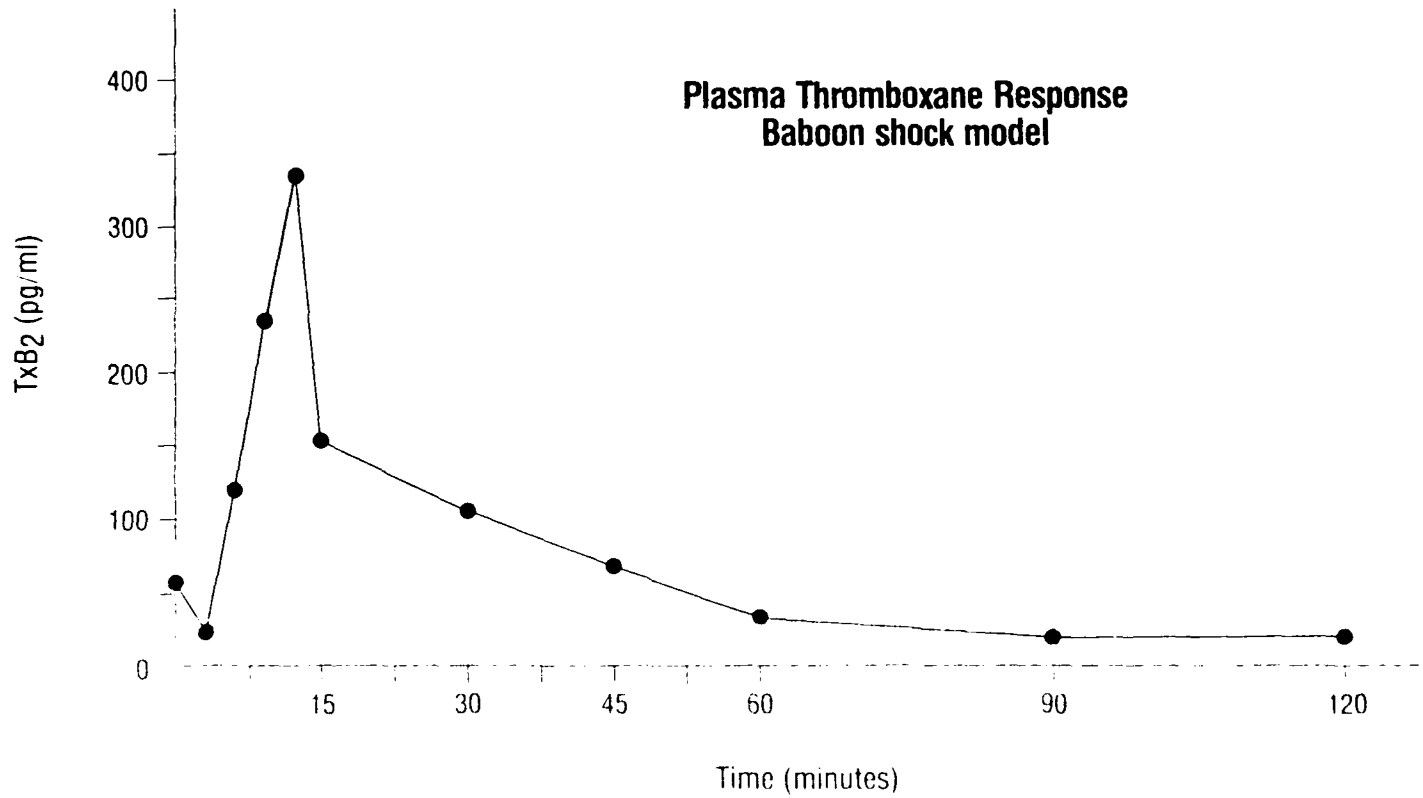

Plasma Thromboxane Response
Baboon shock model
TxB_2 (pg/ml)
400
300
200
100
0
15
30
45
60
90
120
Time (minutes)

Fig. 12

Conclusions

Thromboxane A_2

- Produced in the acute response to hemorrhage.
- Associated with improved survival.
- Does not contribute to the persistent vasoconstriction and maldistribution of blood flow.

Angiotensin II

- Elevated levels of renin are not maintained in this non-resuscitated model.
- The use of a converting enzyme inhibitor to alleviate persistent vasoconstriction is not indicated.

THE EFFECTS OF ADENOSINE TRIPHOSPHATE MAGNESIUM CHLORIDE ($ATP-MgCl_2$) ON SURVIVAL OF RABBITS IN HEMORRHAGIC SHOCK

Summary

ATP-$MgCl_2$, an energy rich molecule, has been shown to improve survivability after hemorrhagic shock. Twenty-five anesthetized New Zealand rabbits underwent shock by exsanguination until mean femoral artery pressure ($\overline{FAP}$) was 60 mmHg for one hour and 40 mmHg for a second hour. Resuscitation with the shed blood followed immediately. Ten rabbits were then treated with 30 mg/kg of ATP-$MgCl_2$ infused at a rate of 0.206 ml/min. Hemodynamics indicate no significant difference between the control and treated groups in heart rate (HR) at any time during the six hour monitoring period. In all rabbits HR was significantly lowered after two hours of shock. At 20 minutes post-ATP-$MgCl_2$ infusion, $\overline{FAP}$ was 36.7 ± 12.2 (mean $\pm$ S.D.) mmHg for the treated group and 83.9 ± 10.0 mmHg for the control. Survival at 72 hours post-shock significantly improved from 33% in the control to 80% in the treated group ($p<0.01$). After one week 27% of the control and 80% of the treated rabbits survived ($p<0.005$). Animals surviving for at least one week were sacrificed. The average survival time was greatly increased from 71.6 ± 64.4 hours in the control to 139.5 ± 60.1 hours in the treated group ($p<0.025$). These results demonstrate the benefit of exogenous ATP-$MgCl_2$ administration in hemorrhagic shock. Although the exact mechanisms for ATP-$MgCl_2$ intervention in shock have not been elucidated, this study is strong evidence for the involvement of ATP-dependent reactions and metabolic factors in shock.

Introduction

Adensoine triphosphate (ATP) is the source of energy for many metabolic reactions. In shock, it has been demonstrated that ATP levels are depleted, presumably because utilization has exceeded production of ATP.[3,8,9,10,13,19,20,22,24,25] Consequently, replacement of the diminished ATP supply seems to be a logical approach to investigating the participation of metabolic factors in shock.

ATP is a chelater of divalent ions, however, when bound to $MgCl_2$ these effects are minimized. ATP-$MgCl_2$ is a vasodilator and is also thought to permeate the cell membrane under ischemic conditions. Exogenous administration of ATP-$MgCl_2$, in essence, provides the cells with the required ATP and avoids many of the side effects.

A number of studies have exhibited enhanced survival with administration of ATP-$MgCl_2$.[1,3,13,15] All of these studies have been performed in rats. Therefore, this present study was undertaken to determine if ATP-$MgCl_2$ significantly improved survival in rabbits from hemorrhagic shock.

Materials and Methods

Surgical and Experimental Procedures

Twenty-five white New Zealand rabbits (1.8-3.0 kg) were initially sedated with ketamine hydrochloride (Ketalar-Parke-Davis) 50 mg/kg intramuscularly. A butterfly (25 gauge) was inserted into an ear vein, and subsequent anesthetization was maintained with pentobarbital sodium (Nembutal Sodium-Abbott) 5 mg/kg. Pentobarbital sodium was not given during the two hour period of hemorrhagic shock. Both femoral arteries and one femoral vein were cannulated with polyethylene tubing (PE-190).

One of the catheters from an artery was connected to a transducer (Statham P-23AA) leading to a four-channel recorder (Hewlett-Packard 7754A). This permitted monitoring of femoral artery pressure throughout the experiment. The catheter from the femoral vein was attached to an IV infusion set containing 5% dextrose in lactated Ringer's solution. A tracheotomy was performed, and intubation with a 2.5-3.5 mm endotracheal tube (Portex-Murphy) followed. The rabbits were placed on a Harvard respirator pump which was adjusted to a tidal volume of 40cc and a respiratory rate of 20 per minute. A left lateral thoracotomy was done at the fourth intercostal space to induce further trauma and in anticipation that microspheres could be injected in future studies. After 5 minutes, the chest was closed. An 8F polyethylene catheter attached to a vacuum pump served as suction for any fluids within the chest. After removing the animals from the respirator, the femoral artery pressure ($\overline{FAP}$) was allowed to stabilize for several minutes.

At this point, hemorrhagic shock was induced by exsanguination from the femoral artery until $\overline{FAP}$ = 60 mmHg over a 10 minute interval (Shock Phase I). This pressure was maintained for one hour by infusion or withdrawal of small amounts of blood. Further exsanguination then proceeded over another 10 minutes until $\overline{FAP}$ = 40 mmHg (Shock Phase II). After an hour at this reduced pressure, the rabbits were resuscitated by returning the shed blood through the venous catheter over a 10 minute period.

Following hemorrhagic shock and resuscitation, ten animals, which comprised an experimental group, received ATP-$MgCl_2$ at a dose of 30 mg/kg. The remaining 15 animals comprised a control group and were not treated after resuscitation. Adenosine 5'-triphosphate in magnesium salt (Sigma Chemical Company) was dissolved in a volume of 0.9% sodium chloride solution such that the final concentration was 30 mg/ml. The resulting solution was

filtered using a Pall Ultipor IV filter/air eliminator (Pall Biomedical Products Corporation). Treated rabbits were administered the ATP-$MgCl_2$ solution intravenously at a rate of 0.206 ml/min over a 10 minute interval by employment of a mechanical infusion/withdrawal pump (Harvard Apparatus Co., Inc.). All animals were monitored for 4 hours after resuscitation. Only a maintenance volume of 5% dextrose in lactated Ringer's was infused during the entire procedure (8-10 ml/hr). After this monitoring period, endotracheal tubes and catheters were removed. Both the tracheal incision and the femoral sites were closed. Rabbits were then returned to their cages and received water and food ad libitum. "Survival" was considered to be survival for at least 72 hours from the onset of hemorrhagic shock.

Statistical Analysis

Hemodynamic and fluid exchange data were analyzed by using the Student's t distribution for two independent samples. The *chi-square* test was employed for the data on percent survival after 72 and 168 hours. The average survival time was compared between the control and treated groups also by the Student's t-test. This value, however, is the lower limit for survival, as animals surviving for one week were sacrificed.

From the data in the first table, it can be demonstrated that the 15 control rabbits and 10 treated rabbits did not differ significantly in terms of weight or fluid exchange. The control group had a mean weight of 2.28 ± 0.6 kg (mean ± 5.0), while the treated group weighed 2.22 ± 0.2 kg. Control rabbits received 63.7 ± 24.5 ml of 5% dextrose in lactated Ringer's solution during the course of the experiment and excreted 95.0 ± 56.1 ml of urine (n=9). Those animals which were treated were given 51.5 ± 12.2 ml of 5% dextrose in lactated Ringer's and concurrently excreted 44.4 ± 39.6 ml of

urine (n=5). Both input and output of fluid was thus not a significant factor. Also relating to fluid exchange is the amount of blood exsanguinated after 2 hours of shock. Control animals had 73.7 ± 25.3 ml of blood withdrawn, while treated rabbits had 77.0 ± 12.6 ml removed. This too was not a significant difference.

The hemodynamics in Table II indicate that the treated and control groups do not differ significantly in $\overline{FAP}$ except at 2½ hours post-shock. At this time, the $\overline{FAP}$ for the control was 83.9 ± 10.0 mmHg, whereas the $\overline{FAP}$ for the treated animals was 36.7 ± 12.2 mmHg ($p<0.001$), as shown in Figure 1. This change in pressure occurred 20 minutes after the total volume of ATP-$MgCl_2$ had been administered. The $\overline{FAP}$ at 2½ hours post-shock for treated animals also denotes a significant decrease from the $\overline{FAP}$ just prior to ATP-$MgCl_2$ infusion, 77.2 ± 14.2 mmHg ($p<0.001$).

Data concerning the heart rate showed no significant difference between control and treated rabbits at any of the times considered. Even at 20 minutes post-ATP-$MgCl_2$ infusion, there was no significant variation in the heart rate. In comparing the heart rate within each group, it appears that there is no significant difference between pre-shock values and shock Phase I values. After 2 hours of hemorrhagic shock, the heart rate was 156.7 ± 18.0/minute for controls and 162.0 ± 24.9/minute for treated. This proved to be significantly reduced from pre-shock levels: 203.0 ± 24.5/minute for the controls ($p<0.001$) and 195.0 ± 17.8/minute for the treated rabbits ($p<0.005$). At 2½ and 6 hours post-shock, the heart rates were also reduced from pre-shock levels. (At 2½ hours, 148 ± 11.0/minute ($p<0.001$) for the control and 123.0 ± 48.8/minute ($p<0.001$) for the treated and at 6 hours, 177.8 ± 25.4/minute ($p<0.05$) and 170.0 ± 32.1/minute ($p<0.05$) for the control and treated, respectively.

Table III contains the data regarding the survival of the rabbits after hemorrhagic shock. At 72 hours post-shock, 80% of the treated rabbits were still living, while only 33% of the control group survived. After 168 hours, the survival rate was still 80% for the treated group and only 27% of the control. Both of these comparisons demonstrated a significant difference, as $p<0.001$ at 72 hours and $p<0.001$ at 168 hours. The average survival time was 71.6 ± 64.4 hours for the control and 139.5 ± 60.1 hours for the treated group. This is also a significant increase in survival time for those receiving ATP-$MgCl_2$ after resuscitation ($p<0.025$).

Discussion

Adenosine 5'-triphosphate (ATP), an energy rich molecule, has pyrophosphate groups which have a high affinity for divalent cations. By using ATP complexed to magnesium chloride ($MgCl_2$), the chelating effect of ATP is minimized. ATP is also a potent vasodilator. $MgCl_2$ affects the circulation as well, however, when ATP is bound to $MgCl_2$ the circulatory changes are slightly different from the separate components.[3] Magnesium also inhibits deamination and dephosphorylation of ATP, thus allowing higher concentrations of ATP to be available when given as ATP-$MgCl_2$.

The vasodilatory effects of ATP-$MgCl_2$ are evident in this experiment, as the $\overline{FAP}$ dropped from 77.2 ± 14.2 mmHg to 36.7 ± 12.2 mmHg after infusion of ATP-$MgCl_2$ in the treated rabbits. Such a dramatic reduction in $\overline{FAP}$ was not observed in the control group.

Vasodilation, however, is not generally believed to be the primary action of ATP-$MgCl_2$ in improving survival after hemorrhagic shock. Chaudry, et. al., demonstrated this theory by administering adenosine diphosphate (ADP) and adenosine monophosphate (AMP), more potent vasodilators than ATP, and showing that

survival was not enhanced.[illegible] ATP-$MgCl_2$ operates by vasodilation to an extent, but it is thought to also have other beneficial effects on microcirculation or regional perfusion.[1,7]

In this experiment, the advantages of administering ATP-$MgCl_2$ immediately after reinfusion the shed blood, has been confirmed. Survival increased significantly from 33% in the control to 80% in the treated group after 72 hours. The average survival time is also indicative of a definitive improvement with ATP-$MgCl_2$.

Several studies have also shown increased survival with ATP-$MgCl_2$.[1,3,13,15] Schloerb, et. al., on the other hand, have demonstrated no significant change in survivability between the control and ATP-$MgCl_2$ treated group.[21] In related studies Lytton, et. al., and Siegel, et. al., emphasize the improvement of renal function when ATP-$MgCl_2$ is utilized after ischemia.[2,4,5] Hepatic ATP levels have also been shown to increase.[13,15]

Most of the hypothesis as to why ATP-$MgCl_2$ may be beneficial in hemorrhagic shock suggest that it can restore ATP-dependent reactions. Machiedo, et. al., found that ornithine metabolism and lactate inhibition could be reversed with ATP-$MgCl_2$ in shock.[12] Lactate production is known to be inhibited during shock (Crabtree effect). Kraven, et. al., have measured lactic acid levels in rats and rats undergoing shock. Shocked rats exhibited decreased lactic acid as expected by the Crabtree effect.[16,18] Perhaps, ATP-$MgCl_2$ is allowing for the production ornithine and lactate, two processes indicative of liver function.

Another ATP-dependent function concerns the sodium-potassium ATPase pump mechanisms. Without ATP, the pumping activity of this enzyme will be lost. Ion transport will be impaired and the cell membrane integrity cannot be maintained; cell death will be inevitable. Day, et. al., have shown that

ion imbalance results from hemorrhagic shock by measuring sodium and potassium levels in red blood cells.[17] Several other groups, as well as Day, et. al., have postulated that ATP depletion in the sodium-potassium ATPase pump may be partially responsible for the harmful effects of shock.[5,10,26] Kreis, et. al., also report small changes in red blood cell sodium and potassium levels during hemorrhagic shock, however, they believe that decreased ATP levels are probably not solely responsible for these changes.[23]

ATP may also be important in glycolysis. In shock, glycolytic enzymes may be denatured or inhibited, or substrates and cofactors may be exhausted; thus decreasing the rate of glycolysis.[9] Markov, et. al., specificially studied a rate-limiting step in glycolysis: the phosphorylation of fructose-6-phosphate to fructose 1.6-diphosphate (FDP). The enzyme, phosphofructokinase that mediates this reaction requires ATP as a cofactor. They administered FDP to dogs in hemorrhagic shock to bypass this reaction in glycolysis and showed that ATP levels were raised to within the normal range. Those dogs not receiving FDP retained the reduced ATP levels. FDP also increased survivability significantly.[19]

ATP-$MgCl_2$ may enhance gluconeogenesis as well. Rhodes determined that in shock, there is decreased high energy phosphate production due to mitochondrial dysfunction. A secondary effect is reduced gluconeogenesis, which can be reversed by ATP-$MgCl_2$ administration. ATP increases synthesis of phosphoenolpyruvate (PEP), an important substrate in gluconeogenesis.[14]

Chaudry, et. al., in a number of studies indicate that ATP-$MgCl_2$ provides cells with a burst of energy necessary to "prime the system".[3-7,25] Others imply similar theories by stating that an initial amount of ATP is essential for increasing the biosynthesis of more ATP. In this way, production of ATP

could parallel its utilization. Cell functions are consequently resumed by balancing "consumption and creation of ATP".[24,25]

One possible mechanism for control of energy production has been inferred by Horpacsy and Schnells.[11] They correlated energy metabolism in hypoxic conditions with lysosomal enzyme release. Changes in mitochondrial energy production are affected by direct action of lysosomal enzymes. By administrating aprotinin, a membrane stabilizer or enzyme-inhibitor, after resuscitation from hemorrhagic shock (with shed blood), Horpacsy and Schnells demonstrated increased ATP production.

Many studies, as well as the present one, support the use of ATP-$MgCl_2$ in hemorrhagic shock. Increased survival or enhanced viability of tissues by various means has been demonstrated. Although many have proposed theories, the exact mechanism as to how ATP-$MgCl_2$ affects hemorrhagic shock has not been elucidated. Nevertheless, the fact that ATP-$MgCl_2$ does restore physiological functions implies that metabolic factors are probably involved in shock. This warrants further investigation to determine what these factors are and how they operate in the event of hemorrhagic shock.

References

1. Chaudry IH, Sayeed MM, Baue AE: Effect of adenosine triphosphate-magnesium chloride administration in shock. Surgery 75;1974:220-7.

2. Lytton B, Vaisbort VR, Glazier WB, Chaudry IH, Baue AE: Improved renal function using adenosine triphosphate magnesium chloride in preservation of canine kidneys subjected to warm ischemia. Transplantation 31;1981: 187-9.

3. Chaudry IH, Sayeed MM, Baue AE: Depletion and restoration of tissue ATP in hemorrhagic shock. Arch Surg 108;1974:208-11.

4. Lytton B, Glazier WB, Cahudry IH, Baue AE: The use of adenosine triphosphate with magnesium chloride in the treatment of post-ischemic renal injury. Trans Am Assoc Genitourin Surg 70;1979:145-7.

5. Siegel NJ, Glazier BW, Chaudry IH, Gaudio KM, Gytton B, Baue AE, Kashgarian M: Enhanced recovery from acute renal failure by the post-ischemic infusion of adenine nucleotides and magnesium chloride in rats. Kidney Int 17; 1980:338-49.

6. Osias MB, Siegel NJ, Chaudry IH, Lytton B, Baue AE: Postischemic renal failure: accelerated recovery with adenosine triphosphate-magnesium chloride infusion. Arch Surg 112;1977:729-31.

7. Chaudry IH, Sayeed MM, Baue AE: Evidence for enhanced uptake of ATP by liver and kidney in hemorrhagic shock. Am J Physiol 233;1977:R83-8.

8. Chaudry IH, Sayeed MM, Baue AE: Differences in the altered energy metabolism of hemorrhagic shock and hypoxemia. Can J Physiol Pharmacol 54; 1976:750-6.

9. Jennings RB, Reimer KA, Hill ML, Mayer SE: Total ischemia in dogs hearts, in vitro: 1. Comparison of high energy phosphate production, utilization, and depletion, and of adenine nucleotide catabolism in total ischemia in vitro vs. severe ischemia in vivo. Circ Res 49;1981:892-900.

10. Reimer KA, Jennings RB, Hill ML: Total ischemia in dog hearts, in vitro: 2. High energy phosphate depletion and associated defects in energy metabolism, cell volume regulation and sarcolemmal integrity. Circ Res 49; 1981:901-11.

11. Horpacsy G, Schnells G: Energy metabolism and lysosomal events in hemorrhagic shock after aprotinin treatment. Circ Shock 7;1980:49-58.

12. Machiedo GW, Ghuman S, Rush BF, Kraven T, Dikdan G: The effect of ATP-$MgCl_2$ infusion on hepatic cell permeability and metabolism after hemorrhagic shock. Surgery 82;1981:328-35.

13. Sharma FP, Eiseman B: Protective effect of ATP in experimental hemorrhagic shock. Surgery 67;1966:66-75.

14. Rhodes RS: Impaired mitochondrial function and gluconeogenesis in late shock. J Surg Res 30;1981:325-30.

15. Kraven T, Rush BF, Ghosh A, Adams-Griffin M: Improved survival and metabolic changes in a rat shock model produced by ATP-$MgCl_2$. Curr Surg 35; 1979:435-7.

16. Kraven T, Rush BF, Slotman GJ, Adams-Griffin M: Permeability of the shocked cell to ATP-$MgCl_2$. Surg Forum 30;1979:7-8.

17. Day B, Friedman SM: Red cell sodium and potassium in hemorrhagic shock measured by lithium substitution analysis. J Trauma 20;1980:52-4.

18. Rush BF, Kraven T, Ghuman SS: Hemorrhagic shock and hepatic cell membrane permeability. Surgery 90;1981:489-92.

19. Markov AK, Oglethorpe N, Young DB, Hellems HK: Irreversible hemorrhagic shock treatment and cardiac pathophysiology. Circ Shock 8;1981:9-19.

20. Chaudry IH, Sayeed MM, Baue AE: Alterations in adenosine nucleotides in hemorrhagic shock. Surg Forum 23;1972:1-3.

21. Schloerb PR, Sieracki L, Botwin AJ, Winblad JM Maguire H: Intravenous adenosine triphosphate (ATP) in hemorrhagic shock in rats. Am J Physiol 240;1981:R52-60.

22. Peitzman AB, Shires GT III, Illner H, Shires GT: Effect of intravenous ATP-$MgCl_2$ on cellular function in liver and muscle in hemorrhagic shock. Curr Surg 37;1981:300-4.

23. Kreis DJ, Chaudry IH, Schleck S, Baue AE: Red blood cell sodium, potassium, and ATP levels during hemorrhagic shock. Surg Res 31;1981:225-31.

24. Chaudry IH, Sayeed MM, Baue AE: Effect of hemorrhagic shock on tissue adenine nucleotides in conscious rats. Can J Physiol Pharmacol 52;1974: 131-7.

25. Chaudry IH, Planer GJ, Sayeed MM, Baue AE: ATP depletion and replenishment in hemorrhagic shock. Surg Forum 24;1973:77-8.

26. Illner H, Shires GT: Changes in sodium, potassium, and adenosine triphosphate contents of red blood cells in sepsis and septic shock. Circ Shock 9;1982:259-67.

Table I - Weight and Fluid Exchange Data

	Control (n=15)*	Treated (n=10)*	P Value
Weight	2.28 ± 0.6 kg	2.22 ± 0.2 kg	NS
Total Blood Exsanguinated	73.7 ± 25.3 ml	77.0 ± 12.6 ml	NS
Total 5% Dext. in Lactated Ringer's	63.7 ± 24.5 ml	51.5 ± 12.1 ml	NS
Total urine output	95.0 ± 56.1 ml (n=9)	44.4 ± 39.6 ml (n=5)	NS

*Unless other stated

Table II - Hemodynamics

		Control (n=15)*	Treated (n=10)*	P Value
$\overline{\text{FAP}}$	Pre-shock (0°)	89.9 ± 6.4 mmHg	91.6 ± 13.7 mmHg	NS
	Shock, Phase I (1°)	60.3 ± 8.4	59.8 ± 2.4	NS
	Shock, Phase II (2°)	40.3 ± 2.4	41.7 ± 3.5	NS
	Post-resuscitation (2°10')	78.6 ± 8.6	77.2 ± 14.2	NS
	2°30' (20' post-ATP-$MgCl_2$)	83.9 ± 10.0	36.7 ± 12.2	$p<0.001$
	3°	86.9 ± 7.9	88.0 ± 20.5	NS
	4°	85.5 ± 9.4	87.9 ± 15.4	NS
	5°	86.2 ± 7.8	85.6 ± 13.1	NS
	6°	89.8 ± 9.3	85.5 ± 10.9	NS
HR	Pre-shock (0°)	203.0 ± 24.5/min (n=9)	195.0 ± 17.8/min	NS
	Shock, Phase I (1°)	185.6 ± 18.1 (n=9)†	187.5 ± 21.2 (n=8)†	NS
	Shock, Phase II (2°)	156.7 ± 18.0 (n=9)††	162.0 ± 24.9†††	NS
	2°30' (20' post-ATP-$MgCl_2$)	148.0 ± 11.0 (n=5)††	123.0 ± 48.8††	NS
	6°	177.8 ± 25.4 (n=9)††††	170.0 ± 32.1††††	NS

* Unless otherwise stated
† NS, compared to pre-shock
†† $p<0.001$, compared to pre-shock
††† $p<0.005$, compared to pre-shock
†††† $p<0.05$, compared to pre-shock

Table III - Survival Data

	Control (n=15)	Treated (n=10)	
Percent survival, 72 hours	33%	80%	$p<0.01$
Percent survival, 168 hours	27%	80%	$p<0.005$
Average survival time	71.6 ± 64.4 hours	139.5 ± 60.1 hours	$p<0.025$

Table I: Weight and Fluid Exchange Data

Urine output and 5% dextrose in lactated Ringer's infusion was over a 6 hour monitoring period. The total amount of blood exsanguinated was determined after 2 hours of hemorrhagic shock (one hour at 60 mmHg and a second at 40 mmHg).

Table II: Hemodynamics

Femoral artery pressure ($\overline{FAP}$) and heart rate (HR) were monitored for 6 hours after the onset of shock. Treated rabbits were administered ATP-$MgCl_2$ 30 mg/kg immediately post-resuscitation with the shed blood over a 10 minute period. The control group was untreated after shock and resuscitation.

Table III: Survival Data

"Survival" was considered to be survival for at least 72 hours after hemorrhagic shock. All rabbits surviving for one week were sacrificed.

THE EFFECTS OF FLUOROCARBON-43 RESUSCITATION

FROM HEMORRHAGIC SHOCK IN RABBITS

Summary

Perfluorochemical compounds have the capacity to transport oxygen and have been used extensively experimentally of blood substitutes. They have also been infused in resuscitation from hemorrhagic shock. Twenty-two anesthetized New Zealand rabbits underwent shock by exsanguination until mean femoral artery pressure ($\overline{FAP}$) was 60 mmHg for one hour and 40 mmHg for a second hour. Fifteen rabbits, comprising the control group, were resuscitated with the shed blood and crystalloid. Resuscitation with a volume of Fluorocarbon-43 (perfluorotributlyamine) Emulsion equal to the amount of shed blood followed in seven treated rabbits. The treated group was further divided: four rabbits received additional 5% dextrose in lactated Ringer's solution (5% DLR) to replace a large urine output engendered by apparent osmotic diuresis (Treated II) and the other three rabbits received only a maintenance volume (8-10 ml/hr) of 5% DLR (Treated I). Hemodynamics indicate a significant reduction in $\overline{FAP}$ immediately in the treated group 63.4 $\pm$ 12.1 as compared with 78.6 $\pm$ 8.6 mmHg (mean $\pm$ S.D.) in the control ($p<0.01$). A decreased $\overline{FAP}$ in the treated groups persisted for the entire 4 hours after resuscitation. The heart rate (HR) was significantly lower in the Treated II group 2 hours after shock (122.1 $\pm$ 17.1/min vs 156.7 $\pm$ 18.0/min in the control, $p<0.005$) and 6 hours after shock (127.5 $\pm$ 29.9/min vs 177.8 $\pm$ 25.4/min, $p<0.01$). Arterial blood gases in treated rabbits suggest profound metabolic acidosis 2 hours after shock (pH 7.074, pO_2 96.8, pCO_2 33.2) and a less severe acidosis 2 hours after FC-43 infusion (pH 7.22, pO_2 201.0, pCO_2 36.8). Hematocrit values in the treated groups decreased from 29.8% before shock to 18.7% 2 hours after shock and 11.6% 1 hour after FC-43 infusion (3 hours post-shock). Hematocrit

increased to 20.8% by 24 hours after shock. Survival at 72 hours decreased significantly from 33% in the control to 0% in both treated groups ($p < 0.005$). The average survival time was also reduced significantly from 71.6 ± 64.4 hours in the control to 24.9 ± hours in the treated rabbits ($p < 0.025$). FC-43 resuscitation from shock induced a significant hypotension and apparent fluid shifts raising hematocrit levels rapidly and had decreasing survival in rabbits.

Introduction

Perfluorochemical compounds are known to have superior capacity to carry oxygen and carbon dioxide. They will carry 40% oxygen, while whole blood in fluorocarbon carries only about 20% oxygen. Carbon dioxide is more than twice as soluble.[1] As a result these perfluorocarbons represent potentially excellent alterations to red cells for blood replacement.

Fluosol-DA is a type of perfluorochemical consisting of an emulsion of perfluorodecalin and perfluorotripropylamine. It has the capacity to transport oxygen and expand the plasma. Fluosol-DA has been shown to improve viability when used in place of blood in resuscitation from hemorrhagic shock.[2,3] It is believed to operate primarily through its oxygen unloading capacity and secondly through its ability to decrease blood viscosity; thus increasing blood flow. Oxygen consumption has been shown to decrease and the oxygen debt is restored with Fluosol-DA.[3]

Fluorocarbon-43 (Oxypherol) is another perfluorochemical: perfluoro-tributylamine, which also has excellent oxygen transport capacity. Studies using FC-43 in resuscitation from hemorrhagic shock have not yet been reported. This study was undertaken to determine whether FC-43 has properties similar to Fluosol-DA in rabbits undergoing hemorrhagic shock.

Materials and Methods

Surgical and Experimental Procedures

Twenty-two white New Zealand rabbits (1.65-3.0 kg) were initially sedated with ketamine hydrochloride (Ketalar-Parke-Davis) 50 mg/kg intramuscularly. A butterfly (25 gauge) was inserted into an ear vein, and subsequent anesthetization was with pentobarbital sodium (Nembutal Sodium-Abbott) 5 mg/kg. Pentobarbital sodium was not given during the 2-hour

period of hemorrhagic shock. Both femoral arteries and one femoral vein were cannulated with polyethylene (PE-190). One of the catheters from an artery was connected to a transducer (Statham P-23AA) leading to a four-channel recorder (Hewlett-Packard 7754A). This permitted monitoring of femoral artery pressure throughout the experiment. The catheter from the femoral vein was attached to an IV infusion set containing 5% dextrose in lactated Ringer's solution. A tracheotomy was performed and intubation with a 2.5-3.5 mm endotracheal tube (Portex-Murphy) followed. The rabbits were placed on a Harvard respirator pump which was adjusted to a tidal volume of 40 cc and a respiratory rate 20 per minute. A left lateral thoracotomy was done at the fourth intercostal space to induce further trauma and in anticipation that microspheres could be injected in future studies. After 5 minutes, the chest was closed. An 8F polyethlyene catheter attached to a vacuum pump served as suction for any fluids within the chest. After removing the animals from the respirator, the femoral artery pressure ($\overline{\text{FAP}}$) was allowed to stabilize for several minutes.

At this point, hemorrhagic shock was induced by exsanguinat[illegible] from the femoral artery until $\overline{\text{FAP}}$ = 60 mmHg over a 10 minute interval (Shock Phase I). This pressure was maintained for one hour b[illegible] infusion or withdrawal of small amounts of blood. Further exsanguination then proceeded over another ten minutes until $\overline{\text{FAP}}$ = 40 mmHg (Shock Phase II). After an hour at this reduced pressure, seven rabbits comprising a treated group were resuscitated by returning a volume of Fluorocarbon-43 Emulsion (FC-43, The Green Cross Corporation) equal to the amount of shed blood. FC-43 was infused through the venous catheter over a ten minute period. The remaining 15 rabbits (the control group) were resuscitated with their shed blood.

The FC-43 Emulsion contains FC-43 (perfluorotributylamine) 25 w/v% and Pluronic F-68 (polyoxpropylene-polyoxyethlene copolymer) 3.2 w/v%. Pluronic F-68 is used as an emulsifier and plasma expander to maintain an oncotic pressure similar to that obtained with blood proteins. The emulsion was supplemented with 2 annex solutions which provided a physiological osmolarity, oncotic pressure and buffer capacity. The final concentrations of the components are as follows:

FC-43	20 w/v%
Pluronic F-68	2.56
KCl	0.034
$NaHCO_3$	0.210
NaCl	0.60
$CaCl_2$	0.028
$MgCl_2$	0.020
Glucose	0.180
Hydroxyethyl - starch	3.0

At the time of FC-43 Emulsion infusion, the treated rabbits were placed on 100% O_2 for 4 hours post-resuscitation. The treated group was further divided such that one group (Treated I, n=3) and the control group received a maintenance volume of 5% dextrose in lactated Ringer's (5% DLR, 8-10 ml/hr) during the entire procedure. The other subgroup (Treated II, n=4) was given volume of 5% DLR equal to the amount of urine output for 4 hours after resuscitation plus a maintenance volume during surgery and hemorrhagic shock periods. Thus in this second treated group, fluid input and output were balanced after infusion of FC-43 Emulsion. If the urine output was less than 8-10 ml/hr, then the maintenance volume of 5% DLR was given.

Hemodynamics (femoral artery pressure and heart rate) were monitored for 4 hours post-resuscitation. An arterial blood gas and hematocrit were taken immediately pre-shock, 2 hours post-shock, and 4 hours post shock. Hematocrits were also taken at 6 hours and 24 hours post-shock in those animals which survived.

After this monitoring period, endotracheal tubes and catheters were removed. Both the tracheal incision and the femoral sites were closed. Rabbits were then returned to their cages and received water and food ad libitum. "Survival" was considered to be survival for at least 72 hours from the onset of hemorrhagic shock.

Statistical Analysis

Hemodynamic and fluid exchange data were analyzed using the Student's t distribution for 2 independent samples. The chi-square test was employed for the data on percent survival after 72 and 168 hours. The average survival time was compared between the control and treated groups also by the Student's t-test. This value, however, is the lower limit for survival, as animals surviving for 1 week were sacrificed.

Results

The data in Tables I, II and VI are expressed for the control group (n=15), Treated I group (n=3), Treated II group (n=4), and combined Treated I/II group (n=7). First of all, the rabbits in the treated group weighed significantly less than those in the control group (1.85 ± 0.15 kg vs. 2.28 ± 0.6 kg, $p<0.025$). All rabbits treated with FC-43 Emulsion (both Groups I and II), on the average weighed significantly less than the control group (1.88 ± 0.15 kg vs. 2.28 ± 0.6, $p<0.05$).

The total amount of blood exsanguinated during hemorrhagic shock did not vary significantly in the control and treated groups (73.7 ± 25.3 ml for the control, 61.3 ± 14.4 ml for the Treated I, and 65.4 ± 13.2 ml for Treated II). The total urine output over the course of the experiment did not change significantly (95.0 ± 56.1 ml for the control, 106.0 ± 29.3 for Treated I, and 162.7 ± 63.5 for Treated II). The total amount of 5% dextrose in lactated Ringer's (5% DLR) solution infused did not vary significantly from the control group (63.7 ± 24.5 ml) to the treated groups (92.1 ± 51.8 ml). Even the Treated II group, in which 5% DLR was infused to balance urine output, did not have a significantly increased 5% DLR intake (118.0 ± 56.2 ml vs 57.7 ± 13.7 ml for the Treated I).

Hemodynamic data in Table II indicate that the initial mean femoral artery pressure ($\overline{FAP}$) does not vary between the control (89.9 ± 6.4 mmHg) and the treated groups (87.8 ± 4.7 mmHg). Shock Phases I and II were manipulated so $\overline{FAP}$ would be 60 and 40 mmHg respectively; thus $\overline{FAP}$ was similar at these times. Immediately after the 10 minute resuscitation period, there was a significant difference between the control group (78.6 ± 8.6 mmHg) and the treated groups (63.4 ± 12.1 mmHg, $p<0.01$). During the entire 4-hour monitoring period after resuscitation, $\overline{FAP}$ was significantly lower in the FC-43 Emulsion treated groups. At 3 hours post-shock, the Treated II group had $\overline{FAP}$ = 62.0 ± 4.0 mmHg while the control group had $\overline{FAP}$ = 86.9 ± 7.9 mmHg ($p<0.001$). The Treated I group ($\overline{FAP}$ = 84.0 ± 6.9 mmHg), however, did not have a significantly lower $\overline{FAP}$ from the control. Again at 4 hours post-shock, the Treated II group ($\overline{FAP}$ = 65.0 ± 2.0 mmHg), but not the Treated I group ($\overline{FAP}$ = 80.0 ± 4.0 mmHg) varied significantly from the control group ($\overline{FAP}$ = 85.5 ± 9.4 mmHg). The mean $\overline{FAP}$s

for the control group seem to be fairly constant after resuscitation and the $\overline{FAP}$ at 6 hours post-shock (89.9 ± 9.3) did not change significantly from the pre-shock value (90.0 ± 6.4 mmHg). For the treated groups, there is a rise in $\overline{FAP}$ from 2 hours to 4 hours post-shock, at which point the $\overline{FAP}$ begins to decrease gradually. At 6 hours post-shock, the $\overline{FAP}$ was 65.7 ± 17.1 mmHg and had decreased significantly from the pre-shock level ($\overline{FAP}$ = 87.7 ± 4.7 mmHg).

The heart rate was not significantly different in the control and treated groups before and 1 hour post-hemorrhagic shock. After 2 hours of shock, the heart rate was significantly reduced from 156.7 ± 18.0/minute in the control group to 134.3 ± 21.5/minute in both treated groups. The Treated I group had a heart rate of 122.1 ± 17.1/minute ($p<0.005$, with the control), while the Treated I group was not significantly reduced (150.0 ± 17.3/minute). At 6 hours post-shock, only the Treated II group with a heart rate of 127.5 ± 29.9/minute was significantly decreased from the control (177.8 ± 25.4, $p<0.01$). Treated II was also significantly less than Treated I (183.3 ± 32.2, $p<0.05$) in heart rate.

Table III displays the results of arterial blood gases taken pre-shock, 2 hours post-shock, and 4 hours post-shock in the treated rabbits only. Before shock the pH was 7.325 ± 0.07. After 2 hours of hypotension, the pH was significantly lowered to 7.074 ± 0.06 ($p<0.001$) and 2 hours later (2 hours after resuscitation), the pH was elevated significantly to 7.220 ± 0.09 ($p<0.01$) but was still significantly lower than the control ($p<0.05$).

The pO_2 was significantly decreased from 85.7 ± 28.8 mmHg just prior to shock and 96.8 ± 20.5 mmHg 2 hours post-shock to 201.0 ± 116.8 mmHg at 4 hours post-shock ($p<0.025$ and $p<0.05$, respectively). The pCO_2 and percent O_2 saturation did not vary significantly during the course of the procedure.

The hematocrit values in Table IV demonstrate a decrease from 29.8 ± 3.9% before shock to 18.7 ± 2.4% after 2 hours of shock ($p<0.001$) and 11.6 ± 4.5% 1 hour after FC-43 Emulsion infusion (3 hours post-shock). At 4 hours post-shock, the hematocrit was 13.0 ± 2.7% and at 6 hours post-shock, it was 17.3 ± 5.0%; both still reduced from the initial value with $p<0.001$. By 24 hours post-shock, the mean hematocrit was 20.8 ± 7.6%, which was no longer significantly different from the pre-shock value of 29.8 ± 3.9%.

Survival data is shown in Table V. At 72 hours, 33% of the control rabbits were living, while all of the treated rabbits had expired ($p<0.005$). By 168 hours, only 27% of the control continued to survive ($p<0.005$) with the treated groups). The average survival time for the control rabbits was 71.6 ± 64.4 hours; this was significantly greater than the treated animals which survived for only 24.9 ± 16.4 hours ($p<0.025$). For the Treated I group the average survival time was 19.7 ± 2.9 hours, a value not significantly different from the Treated I group (28.8 ± 22.1 hours).

Discussion

Data on weight and fluid exchange indicate that there is a slight difference between the control and FC-43 Emulsion treated groups. The rabbits in the Treated II group weighed significantly less; this, however does not affect the fluid exchange data, as the total amount of blood exsanguinated, total 5% DLR infused, and total urine output do not vary significantly from the control to treated groups. The groups are thus largely comparable.

Hemodynamic results demonstrate that the Treated I group did not differ significantly from the control in $\overline{\text{FAP}}$ or heart rate (HR) at any time during the monitoring period. The Treated II group, in which 5% DLR was infused to balance the amount of fluids excreted, had an $\overline{\text{FAP}}$ that was lower than the control, as shown in Figure 1.

Makowski, in resuscitating with Fluosol-DA 20% similarly showed that mean arterial pressure dropped an average of 10 mmHg in a 1 hour period after infusion of Fluosol-Da.[3] Biro, et. al., on the other hand, obtained a significant increase in mean blood pressure from the pre-bleeding period up until 45 minutes post-Fluosol-DA infusion. Dogs in this experiment were then removed from 100% O_2 and placed on 21% O_2 (room air); the mean blood pressure then dropped significantly (from the pre-bleeding levels).[2]

The HR decreased significantly from the control group to the Treated II group at 2 hours and 6 hours post-shock. A drop in HR after 2 hours of shock is expected, as the control rabbits produced a HR of 156.7 ± 18.0/minute vs 203.0 ± 4.5/minute prior to shock. By 6 hours post-shock the HR is still depressed significantly. In the Treated II group, the decrease in HR is more dramatic from 187.5/minute to 122.2/minute ($p<0.005$). Again 6 hours later, the HR is still significantly lower (127.5/minute, $p<0.01$).

With Fluosol-DA, Biro, et. al., claim that HR decreases just after the bleeding period and gradually returns to baseline values during the 45 minute period of hypovolemia. These HR's persist until well after Fluosol-DA infusion.[2] Perhaps the more severe form of hemorrhagic shock in the present study, as well as the use of FC-43 Emulsion instead of Fluosol-DA account for the reduced heart rates.

For the pre-shock, pH = 7.325 and pCO_2 = 34.1 mmHg

$pCO_2 = 40 - 34.1 = 5.9$

$pCO_2 \times 0.008 = 5.9 \times 0.008 = 0.0472$

$pH = 7.4 - pH$

$pH = 7.4 - pH = 7.4 - 0.0472 = 7.3528$

Metabolic acidosis was more severe in control animals then in animals treated with FC-43.

Biro, et. al., reported similar pCO_2 values of 35 mmHg before shock, 33 mmHg after bleeding, and 36 mmHg after infusion of Fluosol-DA. They also observed a dramatic increase in pO_2 when the animals were placed on 100% O_2.[2] In the present study, the pO_2 increased from 85.7 ± 28.8 mmHg before shock to 201.0 ± 116.8 mmHg, 4 hours after shock. The percent O_2 saturation did not appear to vary significantly over the course of the experiment; thus the O_2 carrying capacity of FC-43 is comparable to that of whole blood.

The hematocrit, as shown in Table IV decreases after 2 hours of shock, even before fluorocarbon administration. Removal of blood, addition of a maintenance volume of 5% DLR, and compensation by fluids entering the vascular system can account for the reduced hematocrit. After infusion of FC-43 Emulsion, the hematocrit drops to 11.6 ± 4.5%. From 3 hours post-shock, the hematocrit steadily rises until 24 hours post-shock, when the value is no longer significantly different from the pre-shock value. Red blood cell production in response to low oxygen requires at least 2 days to initiate and 5 days to attain maximum production.[6] Consequently, the increase in hematocrit over 24 hours is probably due to fluid loss which would increase the concentration of red blood cells rather than the RBC production. FC-43 Emulsion may thus be having a diuretic effect.

Finally, the survival data in Table V support the disadvantage of using FC-43 Emulsion in shock. In both treated groups survival was significantly decreased from the control. The average survival time was also significantly reduced.

From this study it is evident that resuscitation with FC-43 Emulsion decreased survival. FC-43 may be having a diuretic effect, causing excessive

removal of fluids beyond the 4 hour monitoring period after shock; thus even attempted balancing of fluid volume during these 4 hours is not sufficient.

FC-43 may also be inhibiting of stimulating certain essential hormones. Matsuki, et. al., demonstrated that Fluosol-DA was able to restore cardiac output, urine output and plasma levels of some hormones. Levels of plasma ADH and renin however, were not renewed.[4] Hormonal changes may also be responsible for the fluid imbalance.

Takiguchi, et. al., reported that Fluosol-DA caused a slight hypocoagulation and hyperfibrinolysis, although the hemostasis was adequate throughout the experiment. Perhaps FC-43 induces similar phenomena.

No previous studies using FC-43 Emulsion in resuscitation from hemorrhagic shock have been reported. Fluosol-DA has been shown to be advantageous for this purpose in several studies[2,3] but evidently FC-43 has properties which are harmful in shock. The exact mechanism whether through diuretic, hormonal, coagulation or other effects has yet to be determined. Nevertheless, this study demonstrates a definite decrease in survival of rabbits from hemorrhagic shock by resuscitation with FC-43 Emulsion.

References

1. The Green Cross Corporation: FC-43 Emulsion: potential uses of perfluorochemical artificial blood for experimental studies in physiology, biology, biochemistry, chemotherapy, toxicology, metabolism, etc. Technical Information Serv. No 3, September 4, 1976.
2. Biron GP, Beresford-Kroeger, Hendry P: Resuscitation of hemorrhage with Fluosol-DA; effects on hemodynamics and myocardial O_2 supply. Proceedings of the IVth International Symposium on Perfluorochemical Blood Substitutes, Kyoto 1978, pp 417-438.
3. Makowski H: The properties of Fluosol-DA infusion in the treatment of hemorrhagic shock. Proceedings of the IVth International Symposium on Perfluorochemical Blood Substitutes, Kyoto 1978, pp 439-448.
4. Takiguchi M, Tsukada S, Oyama T: Effects of Fluosol-DA on blood coagulation-fibrinolysis system in canine hemorrhagic shock. Proceedings of the IVth International Symposium of Perfluorochemical Blood Substitutes, Kyoto 1978, pp. 449-54.
5. Matsuki A, Shiga T, Oyama T: Effects of Fluorocarbon on endocrine and renal functions during hemorrhagic shock in dogs. Proceedings of the IVth International Symposium on Perfluorochemical Blood Substitutes, Kyoto 1978, pp. 455-60.
6. Guyton AC: Textbook of Medical Physiology, sixth edition, W.B. Saunders Company; Philadelphia, 1981, p. 58.

Table I - Weight and Fluid Exchange Data

	Control (n=15)	Treated I (n=3)	Treated II (n=4)	Treated I/II (n=7)
Weight	2.28 ± 0.60 kg	1.92 ± 0.23 kg	1.85 ± 0.15 kg ($p<0.025$)	1.88 ± 0.17 kg ($p<0.05$)
Total Blood Exsanguinated	73.7 ± 25.3 ml	61.3 ± 14.4 ml	65.4 ± 13.2 ml	63.6 ± 12.7 ml
Total 5% Dextrose in Lactated Ringer's	63.7 ± 24.5 ml	56.6 ± 13.7 ml	118.0 ± 56.2 ml	92.1 ± 51.8 ml
Total Urine Output	95.0 ± 56.1 ml (n=9)	106.0 ± 29.3 ml	162.7 ± 63.5 ml	134.3 ± 54.0 ml

The above table compares the control group which underwent 2 hours of hemorrhagic shock and resuscitation with shed blood. Both of the treated groups experienced the identical shock, but with resuscitation with FC-43 Emulsion. The Treated II group was also given a volume of 5% dextrose in lactated Ringer's to equal urine output after resuscitation plus a maintenance volume (8-10 ml/hr) before resuscitation.

Table II - Hemodynamics

		Control (n=15)	Treated I (n=3)	Treated II (n=4)	Treated I/II (n=7)
$\overline{FAP}$	Pre-shock (0°)	89.9 ± 6.4 mmHg	88.0 ± 6.9 mmHg	87.5 ± 3.4 mmHg	87.7 ± 4.7 mmHg
	Shock, Phase I (1°)	60.3 ± 8.4	62.7 ± 2.3	61.0 ± 2.0	61.7 ± 2.1
	Shock, Phase II (2°)	40.3 ± 2.4	40.7 ± 1.2	40.0 ± 1.6	40.3 ± 1.4
	Post-resuscitation (2°10')	78.6 ± 8.6	74.7 ± 4.6	55.0 ± 7.6 ($p<0.001$)	63.4 ± 12.1 ($p<0.01$)
	3°	86.9 ± 7.9	84.0 ± 6.9	62.0 ± 4.0 ($p<0.001$)	71.4 ± 12.7 ($p<0.01$)
	4°	85.5 ± 9.4	80.0 ± 4.0	65.0 ± 2.0 ($p<0.001$)	71.4 ± 8.5 ($p<0.005$)
	5°	86.2 ± 7.8	72.0 ± 8.0	66.5 ± 12.0 ($p<0.01$)	68.9 ± 10.1 ($p<0.001$)
	6°	89.8 ± 9.3	72.0 ± 8.0	61.0 ± 21.7 ($p<0.025$)	65.7 ± 17.1 ($p<0.005$)
HR	Pre-shock (0°)	203.0 ± 4.5/min (n=9)	210.0 ± 30.0/min	187.5 ± 22.2/min	197.1 ± 26.3
	Shock, Phase I (1°)	185.6 ± 18.1 (n=9)	*	167.5 ± 15.0	178.0 ± 26.8
	Shock, Phase II (2°)	156.7 ± 18.0 (n=9)	150.0 ± 17.3	122.1 ± 17.1 ($p<0.005$)	134.3 ± 21.5 ($p<0.05$)
	6°	177.8 ± 25.4 (n=9)	183.3 ± 32.1	127.5 ± 29.9 ($p<0.01$)	151.4 ± 41.0

Mean femoral artery pressure ($\overline{FAP}$) and heart rate (HR) were monitored for 6 hours after the onset of hemorrhagic shock. The shock period was for 2 hours: one hour at $\overline{FAP}$ = 60 mmHg and a second hour at 60 mmHg.

*Information not available

Table III - Arterial Blood Gases

	Pre-shock (n=7)	2 Hrs Post-shock (n=5)	4 Hrs Post-shock (n=7)
pH	7.325 ± 0.07	7.074 ± 0.06 ($p<0.001$ w/pre)	7.22 ± 0.09 ($p<0.05$ w/pre)*
pO_2	85.7 ± 28.8 mmHg	96.8 ± 20.5 mmHg	201.0 ± 116.8 mmHg ($p<0.025$ w/pre)**
pCO_2	34.1 ± 8.6 mmHg	33.2 ± 7.0 mmHg	36.8 ± 6.3 mmHg
% O_2 Saturation	90.1 ± 5.7% (n=5)	84.8 ± 3.3% (n=3)	95.4 ± 6.9% (n=4)

Shown here are arterial blood gas results drawn just prior to shock, 2 hours post-shock and 4 hours post-shock for the animals with FC-43 Emulsion (Treated I and II groups).

*$p<0.01$ with pH at 2 hours post-shock
**$p<0.05$ with pO_2 at 2 hours post-shock

Table IV - Hematocrit Results

	Hematocrit (%)	p Value w/Pre-shock
Pre-shock (n=7)	29.8 ± 3.9%	
2 hrs post-shock (n=5)	18.7 ± 2.4%	p<0.001
3 hrs post-shock (n=4)	11.6 ± 4.5%	p<0.001
4 hrs post-shock (n=7)	13.0 ± 2.7%	p<0.001
6 hrs post-shock (n=5)	17.3 ± 5.0%	p<0.001
24 hrs post-shock (n=4)	20.8 ± 7.6%	NS

The following are hematocrit values for the treated rabbits only. FC-43 Emulsion was infused during resuscitation which occurred at 2 hours post-shock over a 10 minute interval.

Table V - Survival Data

	Control (n=15)	Treated I (n=3)	Treated II (n=4)	Treated I/II (n=7)
Percent survival, 72 hours	33%	0% ($p<0.005$)	0% ($p<0.005$)	0% ($p<0.005$)
Percent survival, 168 hours	27%	0% ($p<0.005$)	0% ($p<0.005$)	0% ($p<0.005$)
Average survival time (hours)	71.6 ± 64.4 hr	19.7 ± 2.9 hr* ($p<0.01$)	28.8 + 22.1 hr ($p<0.05$)	24.9 ± 16.4 hr ($p<0.025$)

Following the 6 hour monitoring period after shock, animals were returned to the cage. Survival was recorded until one week (168 hours) post-shock. Percent survival data were analyzed using the chi-square test.

*Treated I did not differ significantly from Treated II in average survival time.

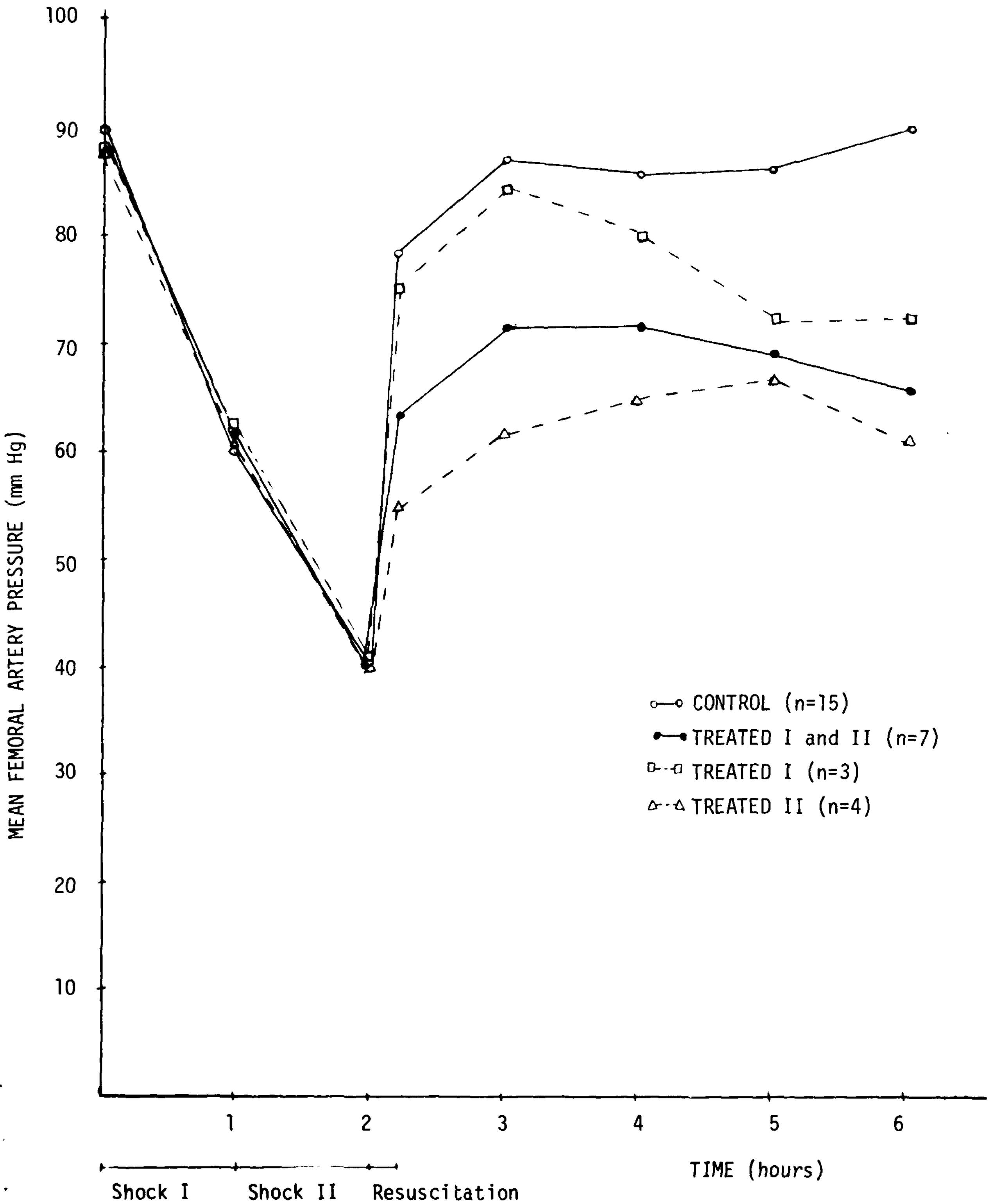
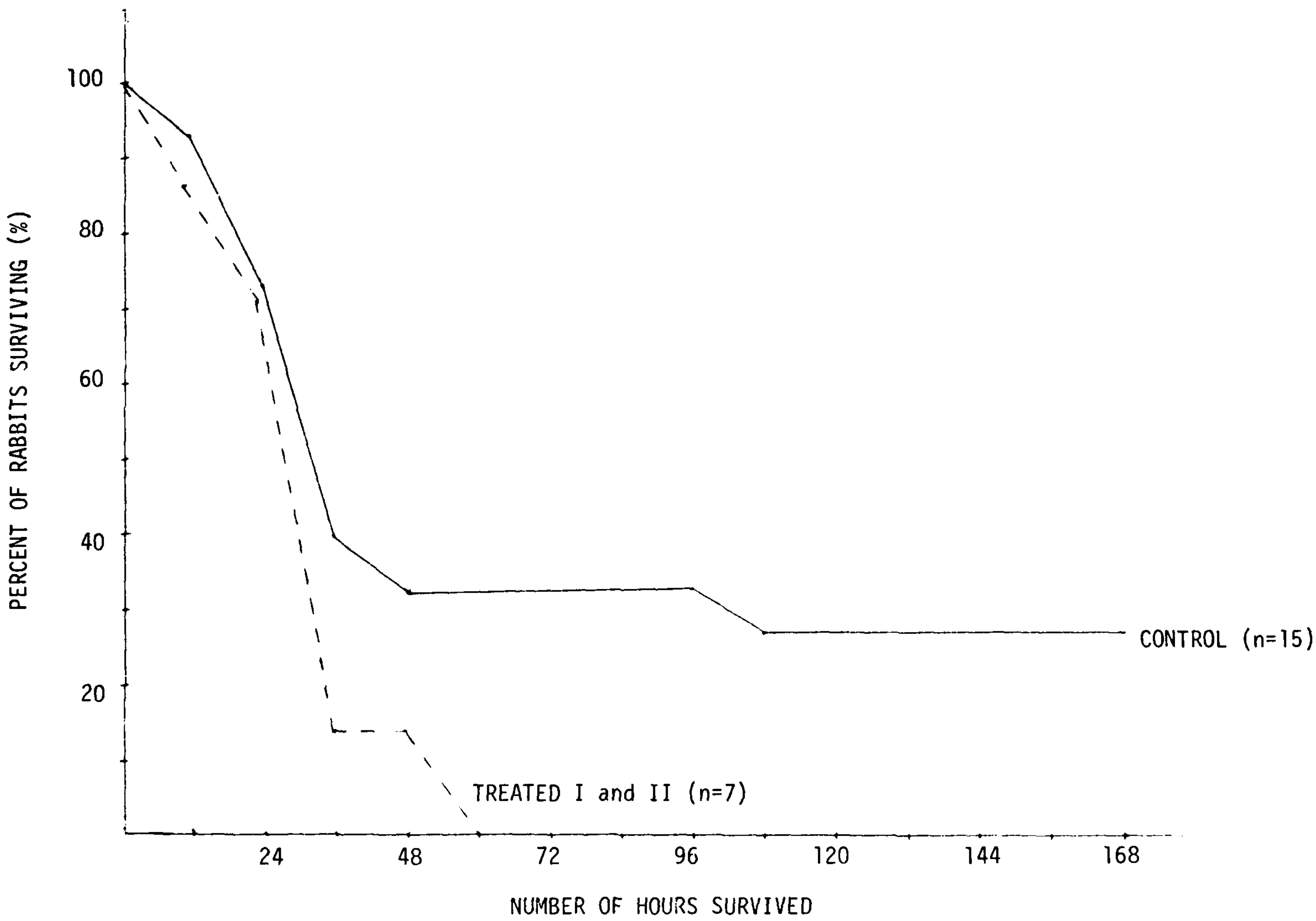


Figure 1: MEAN FEMORAL ARTERY PRESSURE VS. TIME

FIGURE 2: PERCENT OF RABBITS SURVIVING VS. NUMBER OF HOURS SURVIVED



APPENDIX E

THE EFFECTIVENESS OF NALOXONE

ON RABBITS IN HEMORRHAGIC SHOCK

Introduction

It is known that endogeneous opiates are able to significantly depress blood pressure. The cardiovascular system is extremely sensitive to endogeneous opiates such as β-endorphins.[8,16,27] A hypothalamic releasing factor stimulates the release from the pituitary gland of pro-opiocortin, which yields ACTH and endorphins. Endorphins, in turn, may disturb prostaglandin regulation circulatory function.[3,8,31] Certain forms of stress, such as hemorrhagic, septic or cardiogenic shock, may activate release of endorphins which in turn can reduce total peripheral resistance and effect tissue perfusion.[2,3,6] It seems likely that endorphins contribute to hypotension in hemorrhagic shock. Therefore, the blockade of endorphin release might be expected to reduce hypotension. Naloxone, a specific opiate antagonist, has been shown to favorably effect the hypotension of hemorrhagic shock in animals, thus, promoting recuperation by improving cardiac performance. Naloxone decreases heart rate and increases blood pressure, pulse pressure and survival in anesthitized dogs, cats, pigs and rats and conscious rats and rabbits.[2,3,6,8,16,28,31] The demonstration of improved cardiovascular performance and survival in these animals in experimental shock suggests therapeutic value of Naloxone in the treatment of hemorrhagic shock. Since the rabbits have been shown to be good models in previous shock experiments, we chose to use them to test the effect of Naloxone on cardiac performance and survivability.

Materials and Methods

Nineteen white New Zealand rabbits (1.8-3.0 kg) were sedated with ketamine hydrochloride (Ketalar-Parke-Davis) 50 mg/kg intramuscularly. A twenty-five gauge butterfly was inserted into an ear vein and subsequent

anesthetization was through pentobarbital sodium (Nembutal Sodium-Abbott) 5 mg/kg administered intravenously. Pentobarbital sodium was not given during the 2 hour period of hemorrhagic shock. Both femoral arteries and 1 femoral vein were cannulated with polyethylene tubing (PE-190). One of the catheters from the arteries was connected to a transducer (Statham P-23AA) leading to a 4 channel recorder (Hewlett-Packard 7754A). This permitted monitoring of femoral artery pressure throughout the experiment. The catheter from the femoral vein was attached to an IV infusion set containing 5% dextrose in lactated Ringer's solution. A tracheotomy was performed and intubation with a 2.5 to 3.5 mm endotracheal tube (Portex-Murphy) followed. The rabbits were placed on a Harvard respirator pump which was adjusted to a tidal volume of 25 cc and a respiratory rate of 20 per minute. A left lateral thoracotomy was done at the fourth intercostal space. After 5 minutes, the chest was closed with 3-0 mersilene at the level of the ribs, muscles and subcutaneous tissue and 3-0 silk at the skin. An 8F polyethylene catheter attached to a vacuum pump served as suction for any fluids within the chest. After removing the animals from the respirator, the femoral artery pressure ($\overline{FAP}$) was allowed to stabilize for several minutes.

At this point, hemorrhagic shock was induced by exsanguination from the femoral artery until $\overline{FAP}$=60 mmHg over a 10 minute interval. This pressure was maintained by exsanguination or reinfusion of small amounts of blood for 1 hour, after which further exsanguination proceeded until $\overline{FAP}$=40 mmHg. After an hour at this reduced pressure, the 15 control rabbits were resuscitated by returning the shed blood through the venous catheter over a 10-minute period. Four of the animals comprised an experimental group which were administered a (-) Naloxone-HCl bolus at a dose of 10 mg/kg intravenously 1 minute before resuscitation by returning shed blood over a 10-minute period.

Treated rabbits were maintained on (-) Naloxone-HCl at a dosage of 10 mg/kg/hr in 8-10 ml/hr of 5% Dextrose in lactated Ringer's by employment of a mechanical infusion/withdrawal pump (Harvard Apparatus Co., Inc.). The solution was filtered using a Pall Ultipor IV filter/air eliminator (Pall Biomedical Products Corporation). All animals were monitored for 4 hours after resuscitation. Only a maintenance volume of 5% dextrose in lactated Ringer's was infused over this 4 hour period (8-10 ml/hr). After this monitoring period, endotracheal tubes and catheters were removed. The trachea was sutured with 5-0 ethibond and overlying muscle and subcutaneous tissue required 3-0 mersilene. Both the tracheal incision and the femoral sites were closed with 3-0 silk at the skin level. Rabbits were then returned to their cage and received water and food ad libitum. "Survival" was then observed; "survival" was considered to be survival for at least 72 hours from the onset of hemorrhagic shock.

Results

There was no significant difference between test and control animals in any monitored cardiovascular parameters during the baseline period. Similarly, both control and naloxone-treated animals, weighing between 2.0 kg to 2.56 kg, were comparable in terms of volume of blood shed during the shock period. During exsanguination there was a gradual decline in MAP over a 10-minute period to 60 mmHg where animals were maintained for 1 hour. Exsanguination continued to gradually decrease blood pressure to 40 mmHg over a second 10-minute period and it was held at that level for 60 minutes. Exsanguinated blood loss for the 2 groups were 79.27 $\pm$ 2.86 ml/kg for shocked-control rabbits and 77.87 $\pm$ 6.75 ml/kg for the naloxone-treated shocked rabbits.

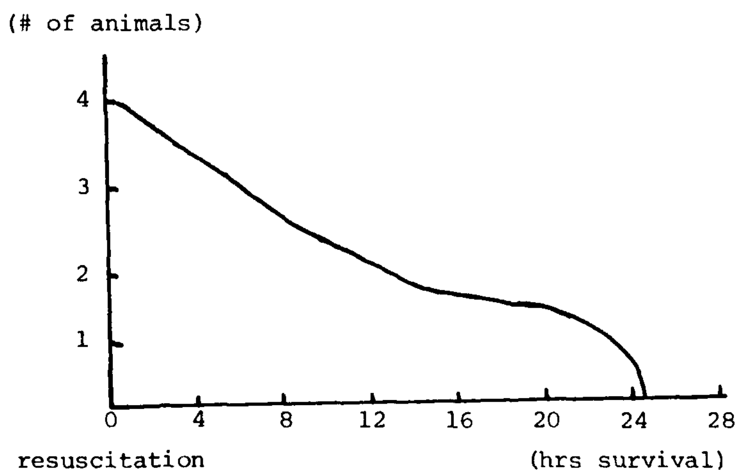
Animals treated with naloxone prior to resuscitation (naloxone-HCl 10 mg/kg) showed no significant increase in MAP. After reinfusion of shed blood, there was no statistical difference between control and naloxone-treated animals initially. However, over the 4-hour post shock monitoring period naloxone-treated rabbits' $\overline{FAP}$ decreased to 57.50 ± 16.52 mmHg (mean SEM) as compared to control rabbits which stablized at 89.70 ± 4.06 mmHg. Survivability was measured over a 72 hour period. All 4 naloxone-treated animals died. Survival time was also considerably shortened (Table 1 and Figure 1).

Table 1 - Survivability

Group	# of Animals	Hrs Survived
Control	5	Survived (72+)
	10	27.69 ± 2.70
With Naloxone	4	17.50 ± 4.65

*Values are expressed as mean SEM for fifteen control and four naloxone-treated animals

Figure 1 - Naloxone-treated Survivability Curve



Discussion

Previous studies have indicated that naloxone, a specific opiate antagonist, reverses the hypotension associated with hemorrhagic shock in various animals under or not under anesthesia.[2,6,7,23,27,28] In our study, our end points were reversal of hypotension and survival of anesthetized rabbits after the injection of naloxone in hypovolemic shock. Despite numerous previous observations in animals of the effect of naloxone on hypotension, there was no significant increase in blood pressure in our study after naloxone infusion in rabbits. We also showed a 100% mortality rate within 25 hours of resuscitation of naloxone-treated animals.

Comparing our study to previous hemorrhagic shock experiments which showed an immediate increase in blood pressure, the procedure by which the shock state was induced in the animals in our experiment differed. The majority of the other hemorrhagic shock experiments placed animals in shock not longer than 30 minutes, whereas in our procedure, rabbits were hypotensive for at least 2 hours. A left thoracotomy was also performed to induce trauma prior to hemorrhage. It may be that beyond a certain stress level the naloxone effect is negligible in reversing hypotension from hemorrhagic shock.

Another speculation is the possibility of some complication due to the interaction between pentobarbiturate and endorphin and/or pentobarbiturate and naloxone[1,10,11,27] in the rabbits. Pentobarbiturate was shown in Schadt and York's experiment to dampen the effects of naloxone in hypotension. However, other experiments have demonstrated a rapid reversal of hypotension by naloxone in anesthetized animals including pigs[27], rats[6] and cats.[7] It may also be that the more endogeneous opiod activity in an animal, the more

effect naloxone would have on a rise in blood pressure.[7] The differencc in levels of response to naloxone by various animals coupled with the complication of anethetics might have caused the lack in blood pressure response to naloxone in our rabbits.

Finally, the apparent higher mortality rate in naloxone-treated rabbits in comparison to the control group may be related to the small number of animals in the treated group. Naloxone treatment was performed on only 4 rabbits whereas there were 15 control animals. A previous rabbit experiment showed a "positive" effect of naloxone on hypotension.[28] Therefore, it could be premature to conclude that naloxone has an adverse effect in rabbits at this point.

In conclusion, further studies are needed in order to reach a probable conclusion on the effectiveness of naloxone as a reversal agent in the hypotension produced by hemorrhagic shock. Such experiments may include the chronic conscious preparations or the use of different anesthetic techniques or a shorter shocking period in the procedure.

References

1. Bouckoert JJ, Heymans C: Physiology (London) 90;1937:59. Lacey CF: Proc Soc Exp Biol Med 29;1932:1974.

2. Curtis MT, Lefer AM: Protective actions of naloxone in hemorrhagic shock. Am J Physiol 239;1980:416-21.

3. Dirksen R, Wood GJ, Nijhuis GMM: Mechanism of naloxone therapy in the treatment of shock: a hypothesis. Lancet March 14, 1981:607-8.

4. Dirksen R, Ohen MH, Wood GJ, Verbaan CJ, Haalebos MMP, Verdouw PV, Nijhuis GMM: Naloxone in shock. LANCET December 20 & 27, 1980: 1360-1.

5. Faden AI, Holaday JW: Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. J Infec Dis 142;1980:229-38.

6. Faden AI, Holaday JW: Opiate antagonists: a role in the treatment of hypovolemic shock. Science 205;1979:317-18.

7. Feeuerstein G, Allam R, Bergman F: Reversal by naloxone of hemorrhagic shock in anephric cats. Eur J Pharmacol 65;1980:93-6.

8. Feuerstein G, Chiueh CC, Kopin IJ: Effect of naloxone on the cardiovascular and sympathetic response to hypovolemic hypotension in the rats. Eur J Pharmacol 75;1981:65-9.

9. Florez J, Mediavilla A: Respiratory and cardiovascular effects of met-enkephalin applied to the ventral surface of the brain stem. Brain Res 138;1977:585-90.

10. Furst Z. Foldes FF, Knoll J: The influence of naloxone on barbiturate anaesthesia and toxicity in the cat. Life Sci 20;1977:921.

11. Gold EM, Ganong WF: in Neuroendocrinology.

12. Gurll NJ, Reynolds DG, Vargish T, Lechner R: Naloxone without transfusion prolongs survival and enhances cardiovascular function in hypovolemic shock. J Pharmacol Exp Ther 220;1982:621-4.

13. Hazinski TA, Grunstein MM, Schlueter MA, Tooley WH: Effect of naloxone on ventilation in newborn rabbits. J Appl Physiol 50;1981:713-17.

14. Hess ML, Smith JM, Eaton LR, Klienman W, Okabe E: Chronic opiate receptor occupation and increased lethality in endotoxemia. Circ Shock 8;1981:313-22.

15. Holaday JW, Faden AI: Naloxone acts at central opiate receptors to reverse hypotension, hypothermia and hypoventilation in spinalc shock Brain Res 189;1980:285-99.

16. Holaday JW, Faden AI: Naloxone reversal of endotosin hypotension suggests role of endorphins in shock. Nature 275;1978:450-1.

17. Holaday JW, Faden AI: Naloxone treatment in shock. Lancet July 1981: 201.

18. Holaday J' 'Amato RJ, Faden AI: Thyrotrophin-releasing hormone improves cardiovascular function in experimental endotoxic and hemorrhagic shock. Science 213;1981:216-18.

19. Ledingham IMCA, Cowan BN, Burns HJG: Prognosis in severe shock: Br Med J 284;1982:443-4.

20. Levaire I, Tseng R, Lemaire S: Systemic administration of endorphin: potent hypotensive effect involving a serotonergic pathway. Prov Natl Acad Sci USA 75:1978:6240-2.

21. Lenz K, Druml W, Gassner A, Hruby K, Kleinberger G, Laggner A: Naloxone in shock. Lancet April 1981:834.

22. Peters WP, Friedman PA, Johnson MW, Mitch WE: Pressor effect of naloxone in septic shock. Lancet March 1981:529-32.

23. Reynolds DG, Gurll NJ, Vargish T, Lechner RB, Faden AI, Holaday JW: Blockade of opiate receptors with naloxone improves survival and cardiac performance in canine endotoxic shock. Circ Shock 7;1980:39-48.

24. Reynolds DG, Lechner RB, Gurll NJ, Vargish T: Opiate receptor blockade improves cardiac performance in hemorrhagic shock.

25. Robinson JA, Knodnycky ML, Loeb HS, Racic MR, Gunnar RM: Endotoxin, prekallikrein, complement and systemic vascular resistance. Am J Med 59;1975:61-7.

26. Rossier J, French ED, Rivier C, Ling N, Guillemin R, Bloom FE: Foot-shock induced stress increases - endorphin levels in blood but not brain. Nature 270;1977:618-20.

27. Saleno TA, Milne B, Jhamandas KH: Hemodynamic effects of naloxone in hemorrhagic shock in pigs. Surg Gynecol Obstet 152;1981:773-6.

28. Schadt JC, York DH: The reversal of hemorrhagic hypotension by naloxone in conscious rabbits. Can J Physiol Pharmacol 59;1980:1208-13.

29. Tiengo M: Naloxone in irreversible shock. Lancet Sept 1980:690.

30. Vargish T, David GR, Nelson JG, Lechner RB, John WH, Faden AI. Naloxone reversal of hypovolemic shock in dogs. Circ Shock 7;1980:31-8.

31. Wuster M, Rudger S, Albert H: Inquiry into endorphinergic feedback mechanisms during the development of opiate tolerance. Brain Res 189; 1980:403-11.

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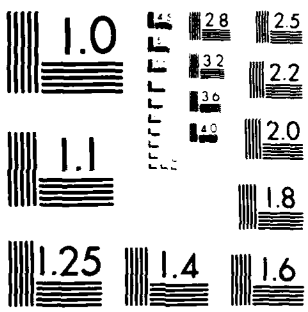

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